THE STUDY OF THE EXPRESSION OF P53 AND KI-67 IN GASTRIC CARCINOMAS AND THEIR CORRELATION WITH CLINICO-PATHOLOGICAL VARIABLES

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CERTIFICATE

This is to certify that this Dissertation entitled "**THE STUDY OF THE EXPRESSION OF P53 AND KI-67 IN GASTRIC CARCINOMAS AND THEIR CORRELATION WITH CLINICO-PATHOLOGICAL VARIABLES**" is the bonafide original work of **Dr. C. HEMA VANEESWARI**, in partial fulfillment of the requirement for M.D., (Branch III) in Pathology examination of the Tamilnadu Dr.M.G.R Medical University to be held in April 2012.

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I, Dr. C. Hema Vaneeswari, solemnly declare that the dissertation titled "**THE STUDY OF THE EXPRESSION OF P53 AND KI-67 IN GASTRIC CARCINOMAS AND THEIR CORRELATION WITH CLINICO-PATHOLOGICAL VARIABLES**" is the bonafide work done by me at Institute of Pathology, Madras Medical College under the expert guidance and supervision of Dr. Sudha Venkatesh, M.D., Professor of Pathology, Institute of Pathology and Electron Microscopy, Madras Medical College. The dissertation is submitted to the Tamilnadu Dr.M.G.R Medical University towards partial fulfillment of requirement for the award of M.D., Degree (Branch III) in Pathology.

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ABBREVIATIONS

GI	:	Gastro - Intestinal
MIB - 1	:	Monoclonal antibody directed against Ki-67 protein
WHO	:	World Health Organization
IL – 1	:	Interleukin - 1
CDH - 1	:	Cadherin – 1 gene
HNPCC	:	Hereditary Non – Polyposis Colorectal Cancer
MLH - 1	:	MutL Homolog – 1gene
hMSH	:	human MutS Homolog gene
hPMS	:	human Protein Homolog gene
OGJ	:	Oesophago – Gastric Junction
MMP	:	Matrix Metallo-Proteinase
TIMP	:	Tissue Inhibitor of Metallo-Proteinase
EGC	:	Early Gastric Carcinoma
IHC	:	Immunohistochemistry
PGP 9.5	:	Protein Gene Product 9.5
PCR	:	Polymerase chain reaction
SSCP	:	Single Strand Conformation Polymorphism
AgNOR	:	Silver stained Nucleolar Organizer Region
PCNA	:	Proliferating Cell Nuclear Antigen
MCM	:	Mini – Chromosome Maintenance
GIST	:	Gastro – Intestinal Stromal Tumour
HRP	:	Horse – Radish Peroxide
LI	:	Labeling Index
AJCC	:	American Joint Committee on Cancer

CONTENTS

INTRODUCTION

INTRODUCTION

Gastric cancer is the third most common cancer in India and the second most common type of cancer worldwide[1]. Approximately 800,000 new cases are diagnosed every year, despite a steadily declining incidence over the previous 50 years[2]. There is wide variation in incidence in different continents. The highest incidence of gastric cancer is in Asia[3,4], Central Europe, and South America. There have also been changes noted in the topographic distribution of gastric cancer in recent years. The incidence of proximal gastric tumors[5,6] has been on the rise. The widespread use of upper GI endoscopy has led to more frequent detection of superficial cancers. This trend has had a dramatic impact on the mortality rate, to the point that gastric cancer is now considered potentially curable when detected at an early stage[7].

Gastric carcinoma exhibits a wide range of morphological phenotypes. The histological appearances of tumor cannot fully reveal the prognosis. The prognosis of gastric carcinoma is mainly dependent on the stage of the disease. Because of the variability of prognosis within a clinical or pathological stage of gastric cancer, there has been a constant search for specific biological markers in order to identify subgroups of patients with more aggressive course of disease[8]. The immunohistochemical protein expression of p53 and Ki-67 has been proposed as a potential tool for the evaluation of the biological behavior of gastric cancer[9].

Mutations of the p53 gene have been found in a number of malignancies[10-17]. In contrast to the normal p53 protein, the mutated p53 protein has an increased half-life and hence accumulates within the cell nucleus. This can be detected immunohistochemically using monoclonal antibodies.

Cell proliferation can be assessed by immuno-histochemical staining with proliferation markers such as Ki-67 antigen. The monoclonal antibody MIB – 1 reacts with nuclear antigen present throughout the cell cycle of proliferating cells but absent in quiescent cells[18]. The level of Ki 67 immuno-reactivity correlates with the degree of tumour proliferation[18].

Patients expressing high levels of p53 and Ki-67 have poorer prognosis because of an aggressive tumour behavior, independent of the already known adverse predictors. Thus the routine evaluation of p53 and Ki -67 could be useful in identifying patients with more aggressive disease and contribute to a better therapeutic approach.

In this study of 50 cases, an attempt is made to study the expression of p53 and Ki-67 immunohistochemically and compare it with various clinico-pathological parameters.

AIMS AND OBJECTIVES

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1. To identify the incidence and distribution of gastric carcinoma in patients admitted in Government General Hospital, Chennai during the year 2010.
2. To study the histo-morphological features of gastric carcinoma including tumour size, tumour location, macroscopic appearance, histological type, grade, depth of infiltration, lymph node status, stage , lympho-vascular invasion, perineural infiltration, lymphocytic response, and necrosis.
3. To study the immunohistochemical expression of p53 in gastric carcinoma
4. To study the immunohistochemical expression of Ki-67 in gastric carcinoma
5. To determine the correlation of p53 and Ki 67 expression with known prognostic factors such as tumor size, histological type, grade, depth of infiltration, lymph node status, stage, presence of tumor necrosis, lymphocytic response, lympho-vascular invasion and perineural infiltration.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Gastric carcinomas are a group of malignant tumours of the stomach arising from the gastric glandular epithelium.

The first case of gastric cancer was reported in the Ebers papyrus in 1600 BC and in the Hippocrates reports related by Galen in the second century AD in Rome[19]. At the end of the first millennium AD, a possible description of a gastric cancer could be read in Avicenna's Medical Encyclopedia. Despite this, in the eighteenth century, gastric cancers were largely unknown because benign and malignant gastric ulcers were only described later by J. Cruveilhier, in 1835.

The official history of gastric cancer surgery began 40 years later when Jules Emile Pean, a very famous French surgeon, performed the first gastric resection for cancer in 1879[20]. The first successful subtotal resection with gastro-duodenal anastomosis was performed by Theodor Billroth in 1881 in Vienna[21].

Later several classification systems were proposed. One of the earliest was the Lauren classification proposed in 1965 which divided gastric adenocarcinomas into 2 types – Intestinal and Diffuse[22.] This was followed by the Ming classification in 1977 which divided the adenocarcinomas into 2 types – expanding and infiltrative, based on the growth pattern[23]. The WHO

classification was proposed in 1977 which was based on the histomorphology[24] (Annexure II).

Epidemiology:

In 2000, about 880 000 people were diagnosed with gastric cancer and approximately 650 000 died of the disease world wide[2]. Japan and Korea have the highest gastric cancer rates in the world[25,26]. Age-standardized incidence rates in Japan are 69.2 per 100,000 in men and 28.6 per 100,000 in women[27].

In India there is a wide variation in the incidence of gastric carcinoma. According to the study conducted by the National Cancer Registry Programme of India in 2001, the number of new gastric cancer cases was estimated to be approximately 35,675 (n=23,785 in men; 11,890 in women)[28]. The incidence rate of gastric cancer was four times higher in Southern India compared with Northern India[29].The rates in rural population were much lower than those in the urban population. Among the six registries in Southern India, the highest incidence in both sexes was reported from Chennai. The age-standardized incidence rates in Chennai are 13.6 per 100,000 in men and 6.5 per 100,000 in women[28].

The 5 year survival rate of early gastric cancers is higher (upto 95%) when compared to those of advanced gastric cancer (10% -20%)[30].

Clinical presentation:

The symptoms associated with gastric cancer are usually non-specific. Early gastric cancers are usually asymptomatic. Some of them may cause anorexia, weight loss, fatigue, nausea, vomiting and mild to moderate epigastric distress. Hematemesis occurs in 10% to 15% cases. Proximal gastric tumours cause dysphagia and distal gastric tumours may cause gastric outlet obstruction.

Pathogenesis:

Gastric carcinogenesis is a multistep and multifactorial process that in many cases appears to involve a progression from normal mucosa through chronic gastritis, atrophic gastritis and intestinal metaplasia to dysplasia and carcinoma[31].

Risk factors:

The risk factors associated with gastric carcinoma include chronic atrophic gastritis, Helicobacter pylori infection, diets rich in salt (dried and salted fish) and low in micronutrients (vitamin C), intestinal metaplasia, smoking, pernicious anemia, bile reflux in patients with post-operative gastric stumps, Menetrier's disease and peptic ulcer disease[32,33]. First-degree relatives of affected patients are almost three times as likely to develop the disease as the general population. This may be partly attributable to H. pylori infection being commoner in families, and the potential role of IL-1 gene polymorphisms.

Etiology:

Diet:

The most consistent etiological factor associated with gastric cancer is diet. Intraluminal and intramucosal synthesis of carcinogens like N-nitrosamines by bacteria[34] and excessive salt which acts as an irritant[35-37], cause inflammation and intestinal metaplasia which later leads to malignancy. Consumption of fresh fruits and vegetables which contain Vitamin C, Vitamin E and carotenoids[38] counteracts the formation of N-nitroso compounds[39] and scavenges oxygen free radicals, thereby playing a protective role.

Helicobacter pylori infection:

Several epidemiological investigations have found a consistent association between H.pylori seropositivity and risk of gastric cancer[40,41]. The development of severe gastritis with atrophy and intestinal metaplasia is particularly associated with infection by CagA-positive strains of the bacillus[42,43] and these strains have been associated with increased risk of gastric cancer in some studies[44]. The sequence of events include development of atrophic gastritis, intestinal metaplasia, dysplasia and carcinoma. The various mechanisms proposed are increased epithelial cell proliferation with a resultant increased risk of mutations[45], bacterial overgrowth with increased potential to generate intraluminal carcinogens[46], increased free radicals[47] and reduced gastric antioxidant levels[48].

Genetic predisposition:

There is evidence that germline truncating mutations in the gene for E-cadherin (CDH-1), a calcium-dependent cell adhesion protein, are responsible for a rare autosomal dominant inherited form of gastric carcinoma in young persons. This condition is characterized by multiple tumours of diffuse or signet ring cell histological types that do not arise in a background of intestinal metaplasia[49]. Affected family members can be identified by mutation-specific genetic testing and offered prophylactic gastrectomy[50]. Patients with hereditary non-polyposis colorectal cancer (HNPCC), which results from germline mutation of one of the DNA mismatch repair genes hMLH1, hMSH2, hMSH6, hPMS1 and hPMS2 also have an increased frequency of gastric cancers[51]. Peutz – Jegher's syndrome also shows an increased risk of gastric cancers[52].

Topography of gastric carcinoma

Carcinomas of the distal stomach are most common in the prepyloric region, in the pyloric antrum and on the lesser curvature. Tumours arising at the cardia in the region of the oesophago-gastric junction (OGJ), whose frequency is increasing are generally smaller than those of the distal stomach. In 1996, Siewert et al proposed a classification of gastro-oesophageal junction adenocarcinomas based upon their location relative to the gastro-oesophageal junction. The tumours whose centre lay between 5 cm proximal to and 5 cm distal to the gastro-oesophageal junction were considered to be oesophago-gastric junction tumours. Siewert et al subdivided these gastro-oesophageal junction cancers into type I if the tumour centre lay 1–5 cm proximal to the

gastro-oesophageal junction, type II if between 1 cm proximal and 1 cm distal to the junction and type III if 1–5 cm distal to the junction[53]. This classification has been internationally recognised and is used by surgeons to plan management of the tumour.

Early gastric cancer:

Early gastric cancer is defined as a carcinoma which is limited to the mucosa or the mucosa and submucosa only, irrespective of the lymph node status. It can be subdivided further after histological examination into two groups, intramucosal and submucosal carcinoma. The term ‘early’ does not imply a stage in the genesis of the cancer but means that the gastric cancer is potentially curable[54]. Early gastric cancer is also known as superficial spreading carcinoma[55], surface carcinoma[56] and cancer gastrique au début[57]. Increasing numbers of early gastric cancers are being detected mainly due to screening programs in countries like Japan. The mean age at presentation is somewhat lower[58] and the duration of symptoms is generally longer[59].

Advanced gastric cancer:

Advanced gastric cancer is defined as a carcinoma which has spread beyond the submucosa into the muscularis propria and beyond, irrespective of the lymph node status. The term ‘advanced’ does not indicate a higher stage of disease but means that treatment of such tumours is difficult and has decreased survival rates.

Macroscopic appearance of gastric cancer:

A sub-classification of the gross appearance of early gastric cancer was devised by the Japanese Gastro-enterological Endoscopic Society on the basis of macroscopic appearances at endoscopy and in gastrectomy specimens. They were divided into three main types and three subtypes. (Figure 1)

Type I - Protruded - The tumour projects clearly into the lumen and includes all polypoid, nodular and villous tumours.

Type II – Superficial - This is further subdivided into three groups:

Type IIa - Elevated above surrounding mucosa by few millimetres.This is seen as a well – circumscribed flat plaque.

Type IIb - Flat. No abnormality is macroscopically visible

Type IIc - Depressed. The surface is slightly depressed below adjacent mucosa

Type III – Excavated - Ulceration of variable depth into the gastric wall.

EGC is located mainly in the corpus and antrum of the stomach[60] . Lesions are multifocal in up to 14% of cases[61].

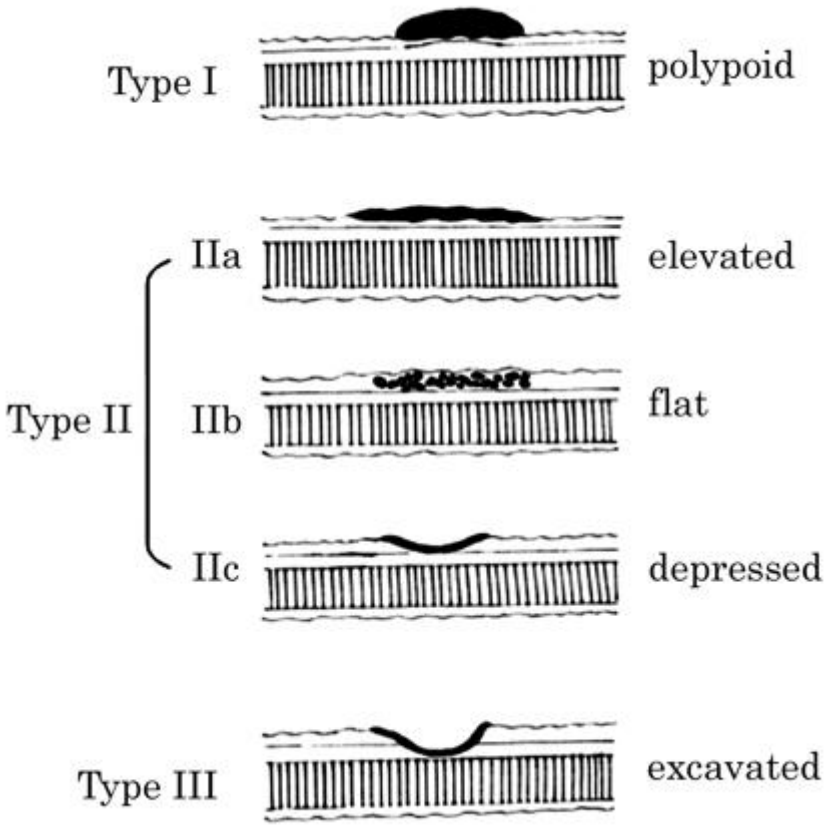


Figure 1 : Gross classification of early gastric cancer

Macroscopic types of advanced gastric cancer can be understood from the schema depicted in 1925 by Dr. R. Borrmann, who was a German surgeon and pathologist. (Figure 2)

Type I – Polypoid / Nodular

Type II – Ulcerative, localized / Fungating

Type III – Ulcerative, infiltrative

Type IV – Diffusely infiltrative

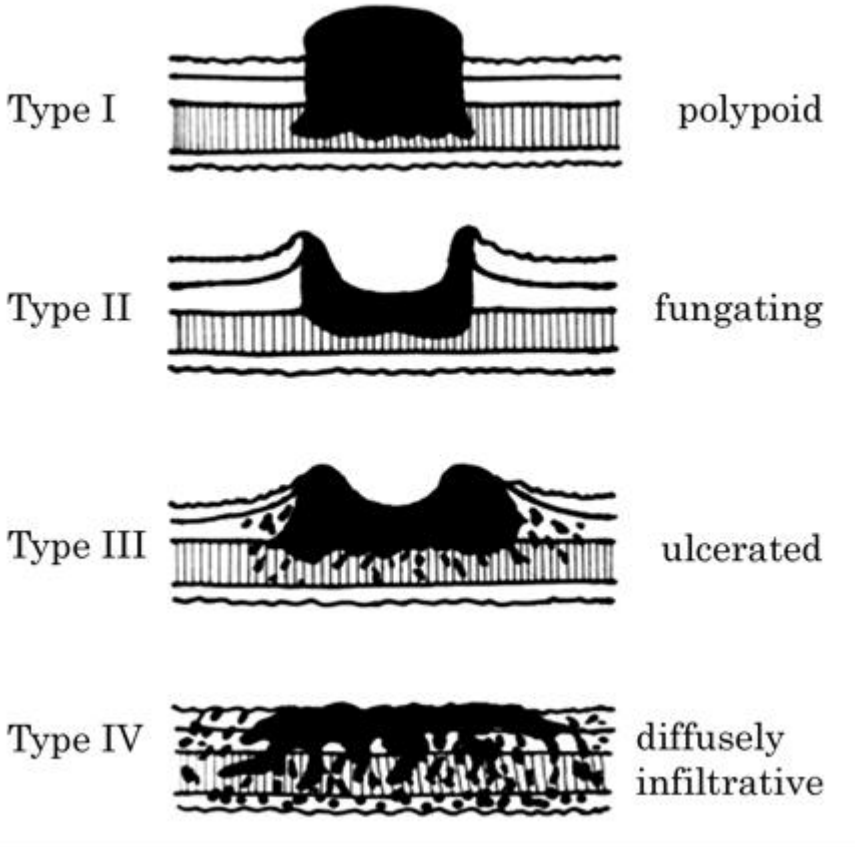


Figure 2 : Borrmann classification of gross types of advanced gastric cancer

Ulcerated tumours occur most frequently in the antrum on the lesser curve and these ulcers are large with an irregular margin, raised rolled edges and necrotic shaggy base[62]. Polypoid, fungating and nodular tumours tend to occur in the body of the stomach in the region of the greater curvature, posterior wall or fundus. Infiltrative cancers spread superficially in the mucosa and submucosa producing plaque-like lesions. It is commonly accompanied by thickness of the entire stomach wall producing the so-called linitis plastica or ‘leather bottle’ stomach. Many gastric carcinomas secrete considerable amounts of mucin which gives the gelatinous appearance of colloid carcinomas.

Gastric adenocarcinomas are either gland-forming malignancies composed of tubular, acinar or papillary structures, or they consist of a complex mixture of discohesive, isolated cells[63]. Several classification systems have been proposed, including Ming, Carniero, and Goseki, but the most commonly used are those of WHO[64] (Annexure II) and Laurén.

WHO CLASSIFICATION:

Tubular adenocarcinoma :

Tubular adenocarcinoma is composed predominantly of neoplastic tubules often showing irregular branching and anastomosis embedded in or surrounded by fibrous stroma. Individual tumour cells are columnar, cuboidal, or flattened by intraluminal mucin. The degree of cytological atypia varies from low to high-grade. A poorly differentiated variant is sometimes called solid carcinoma. An oncocytic variant of tubular adenocarcinoma has been described[65].

Papillary adenocarcinoma :

These are well-differentiated carcinomas with elongated finger-like processes lined by cuboidal cells supported by fibro-vascular connective tissue cores. Some

tumours show tubular differentiation (papillotubular). Rarely, a micropapillary architecture is present. Typically this tumour grows as a polypoid mass into the lumen of the stomach.

Mucinous carcinoma :

WHO defines carcinomas containing large amounts of extracellular mucin in more than 50% of the tumour as Mucinous carcinomas. In some such tumours the cells form glands lined by columnar mucus-secreting cells (well differentiated type). In others there are disaggregated ribbons or clusters of cells which appear to be floating in lakes of mucin (poorly differentiated type). There may also be mucin in the inter-glandular stroma. Scattered signet-ring cells, when present, do not dominate the histological picture. They most commonly occur as polypoid, fungating or ulcerative masses.

Signet ring cell carcinoma:

WHO defines this tumour as "Carcinomas composed predominantly of single cells or small clusters of cells containing intra-cytoplasmic mucus vacuoles and accounting for more than 50% of the tumour". The cells contain nuclei which push against cell membranes creating a classical signet ring cell appearance due to an expanded, globoid, optically clear cytoplasm. These contain acid mucin and stain with Alcian blue at pH 2.5. They also contain cells with no mucin and cells with eosinophilic granular cytoplasm containing neutral mucin. These tumours are more common in younger patients and in the distal stomach. Signet ring cell carcinomas tend to infiltrate the wall of the stomach diffusely and are accompanied by marked fibrosis giving rise to the linitis plastica appearance on gross examination.

LAUREN CLASSIFICATION :

The histological classification of Lauren[22] (1965) divides gastric adenocarcinoma into two main types - Intestinal and Diffuse. Tumours that contain approximately equal quantities of intestinal and diffuse components are called mixed carcinomas. Carcinomas too undifferentiated to fit neatly into either category are placed in the indeterminate category.

Intestinal carcinoma:

Intestinal-type tumours have a glandular pattern usually accompanied by tubules papillary formation or solid components. The glands range from well differentiated to moderately differentiated grade, sometimes with poorly differentiated tumour at the advancing margin. The glandular epithelium consists of large pleomorphic cells with large hyperchromatic nuclei often with numerous mitoses. The adjacent gastric mucosa often shows chronic gastritis, widespread intestinal metaplasia and sometimes dysplasia. Intestinal-type tumours are commoner in the elderly and in males.

Diffuse carcinoma:

Diffuse-type carcinomas are predominantly composed of poorly cohesive diffusely infiltrating small tumour cells with indistinct cytoplasm and hyperchromatic nuclei. Glandular formation may occur in the more superficial part of the tumour. Signet ring cells are common and there may be extracellular mucin in the stroma. Desmoplasia is more pronounced and generally there is no

accompanying intestinal metaplasia or dysplasia. The diffuse tumours usually occur at a younger age and there is equal sex incidence.

CLASSIFICATION OF MING :

The Ming's classification (1977) divides gastric adenocarcinomas into two types - Expanding type and Infiltrating[23]. The expanding type has a pushing edge and forms discrete tumour nodules. This compares roughly to the intestinal type of Lauren and occurs in patients over 50 years of age. The infiltrative type is ill defined and contains widely infiltrative tumour cells with poor inflammatory cell response and collagenous stroma and is more common under the age of 50.

CLASSIFICATION OF MULLIGAN AND REMBER :

This classification expands Lauren's classification by adding a third type - pylorocardiac gland carcinoma[66]. Pylorocardiac carcinomas commonly present as well demarcated fungating tumours. These tumours are commoner in men and are characterized microscopically by varying-sized glands showing tubular or papillary pattern cells that often show striking vacuolation or clear cell change and stain brilliantly with the periodic acid–Schiff reaction.

THE GOSEKI CLASSIFICATION :

Goseki et al divided gastric cancer into four histological types according to the degree of tubular differentiation and the amount of intracellular mucin[67].

Group I - consists of well differentiated tubules with poor intracellular mucin.

Group II - consists of well differentiated tubules & plentiful intracellular mucin.

Group III – consists of poorly differentiated tubules & poor intracellular mucin;

Group IV - consists of poorly differentiated tubules & plentiful intracellular mucin.

CARNEIRO CLASSIFICATION :

Carneiro et al proposed a much simpler system in which the tumours are divided into glandular, isolated cell carcinomas, solid variety and a mixed type that consists of a mixture of glandular and isolated cell types[63].

The rare variants of gastric carcinoma include Adenosquamous carcinoma[68], Squamous cell carcinoma[69], Hepatoid adenocarcinoma[70], Choriocarcinoma[71], Medullary carcinoma with lymphoid stroma[72], Small cell carcinoma[73], Parietal cell carcinoma[74], Gastric carcinoma with rhabdoid features[75] and Carcinosarcoma[76].

SPREAD OF GASTRIC CANCER

Gastric cancer may spread directly through penetration of the serosa and infiltration into organs like pancreas, liver, spleen, transverse colon and omentum and this is particularly common in signet ring cell carcinomas and diffuse carcinomas. The incidence of lymphatic spread increases with increasing depth of invasion into the stomach wall. The nodes commonly involved include the nodes along the left gastric, common hepatic, coeliac arteries and the pancreatic and splenic nodes. More distant lymphatic spread may involve para-aortic and mesenteric nodes. Spread by way of the thoracic duct to the left supraclavicular nodes (nodes of Troisier and of Virchow) is not common.

Hematogenous spread occurs most commonly to the liver, followed by lung, peritoneum, adrenal glands, skin and ovaries (Krukenberg tumour). Diffuse tumours tend to involve unusual sites such as kidney, spleen, uterus and meninges more often[77].

STAGING OF GASTRIC CANCER :

The TNM staging system[78] (Annexure III) is widely used in western countries. It is the best available predictor of prognosis and is recommended.

PROGNOSIS:

The prognosis of gastric carcinoma varies from country to country with Japan having the best results with an overall 5-year survival rate of 46% for advanced carcinoma and 89% for early carcinoma[79]. The overall survival rate in the Western countries is between 4% and 13%[80]. This can be explained at least partly by the greater frequency of superficial carcinomas, aggressive Japanese surgical approach to treatment with extensive and meticulous lymph node dissection[81]. A recent study of untreated early gastric cancer has indicated a 63% cumulative 5-year risk of progression to advanced cancer[82].

PROGNOSTIC FACTORS:

Prognostic factor is defined as any variable that provides information useful in assessing the outcome at the time of diagnosis of the disease. The prognostic factors are classified as clinical factors, morphological factors and genetic / molecular factors. The clinical factors with poor prognosis include younger age group, larger tumor size, and proximal gastric cancers[80]. The 5-

year survival rates in tumours of the cardia are under 20%[83] and the median survival is about 7 months only[84]. The pathological factors play a more useful role in assessing prognosis which includes the following:

1. **Tumour stage**: This parameter is the most significant prognostic factor. One of the features that it incorporates is the depth of the invasion, for the deeper the penetration, the greater the chance of metastasis. This feature is directly related to the gross appearance of tumour – large intraluminal neoplasms have lower incidence of metastasis than those growing primarily within the wall.
2. **Microscopic type and grading**: The intestinal type tumours in Lauren's classification behave relatively better than the diffuse types[85].
3. **Regional lymph node involvement**: With nodal involvement the 5-year survival rate drops to less than 10% when compared to 50% in the node negative cases. The number of nodes involved is also prognostically significant. The overall survival rate declines as the number of positive node increases[86].
4. **Tumour size**: Small tumour size is associated with a better prognosis but this is closely linked to the depth of penetration[79].
5. **Perineural invasion** is associated with poor prognosis when compared to the negative cases.

6. **Lymphatic invasion** is a poor prognostic factor strongly associated with the presence of lymph node metastasis and poor patient survival.
7. **Vascular invasion** denotes the infiltration of tumor cells into vascular spaces and it predicts the risk of recurrence and visceral metastasis.

Other factors reported to have poor prognosis includes tumour necrosis, infiltrative tumour margins and positive surgical margins.

Many molecular biomarkers have been identified which play a significant prognostic role in gastric carcinoma management. DNA aneuploidy has been reported in approximately 40–50% of gastric carcinomas and it has been found that aneuploid tumours are significantly associated with both lymph node and distant metastases and lower survival rates in comparison with diploid cancers[87]. Her 2 neu is a transmembrane epidermal growth factor receptor protein also known as c erb2. Its overexpression is reported to have poorer outcome[88]. Mutation of the *p53* gene was identified in approximately 25% of gastric carcinomas and this correlated well with demonstration of p53 protein overexpression by immunohistochemistry in these tumours. Some studies based on immunohistochemistry indicate that p53 protein overexpression is associated with shortened survival[89] but some studies failed to confirm this[90]. E-cadherin is a transmembrane protein which plays an important role in maintenance of intercellular connections. Germline mutations of the E-cadherin gene (*CDH-1*) are associated with cancers of diffuse type and are highly aggressive[91]. Other factors like increased expression of cathepsin D, p27kip1, increased

proliferation indices and loss of Fhit protein are associated with reduced survival.

p53 :

p53 was identified in 1979 by Lionel Crawford, David P. Lane, Arnold Levine, and Lloyd Old. The human TP53 gene was cloned in 1985. Its character as a tumor suppressor gene was revealed in 1989 by Bert Vogelstein. p53 gene is considered “Guardian of the genome” and represents a tumor suppressor gene located on the 17p chromosome, coding a protein of 53 kD. The role of p53 is central in cell – cycle regulation, in DNA repair and in cell apoptosis. The production of p53 is increased in response to cellular insults or DNA damage and p53 then induces cell - cycle arrest at the G1/S junction. Therefore, p53 is essential for control of tumor growth, apoptosis and maintaining genome stability. Unlike normal p53 protein, which is rapidly removed from the nucleus, mutant forms have a prolonged half-life, which favors intranuclear accumulation, becoming detectable immuno-histochemically. Mutations of the p53 have been observed in a wide variety of human carcinomas, such as colorectal carcinoma, breast carcinoma, gallbladder carcinoma, esophageal carcinoma, and gastric carcinoma. Numerous studies have reported the correlation between the overexpression of p53 and the poor prognosis of patients with these tumors. The p53 pathway is also involved in regulating the metastasis-associated genes, including Maspin, integrin, matrix metallo-

proteinase-2 (MMP-2), MMP-13 and the tissue inhibitor of metalloproteinase-3 (TIMP3).

Mutation of the p53 gene was identified in approximately 25% of gastric carcinomas and this correlated well with demonstration of p53 protein overexpression by immuno-histochemistry in these tumours. Carcinomas of the cardia showed mutation of p53 in a considerably higher proportion of cases than carcinoma of the body or antrum[96]. Overall prevalence of p53 immunoreactivity in advanced gastric carcinoma is about 50–60%. Alterations of the p53 gene have also been demonstrated in precancerous lesions of the stomach. The p53 gene mutation and overexpression of gene protein is more common in intestinal-type carcinomas than in diffuse tumours[97]. Some studies based on immuno -histochemistry indicate that p53 protein overexpression is associated with shortened survival but few other studies have failed to confirm this[89,90].

The most commonly used methods for detection of these mutations are immunohistochemistry, flow-cytometry, polymerase chain reaction-single-strand conformation polymorphism (PCR – SSCP) and genomic sequencing. Although sequencing is the most unambiguous method, it is technically cumbersome. Therefore, both immune-detection and PCR- SSCP have been widely used as alternative methods.

Immuno-histochemically, a positive reaction is considered in the presence of brown immunostained nuclei.

p53-negative (-): Absence of immunostaining in < 10% of the tumour nuclei

p53-positive (+): Presence of immunostaining in > 10% of the tumour nuclei

Ki-67:

Ki-67 also known as MKI67 is a protein encoded by the MKI67 gene[98] which was discovered by Gerdes. The Ki-67 protein was originally defined by the prototype monoclonal antibody Ki-67, which was generated by immunizing mice with nuclei of the Hodgkin lymphoma cell line L428. The name is derived from the city of origin (Kiel, Germany) and the number of the original clone in the 96-well plate.

Ki-67 is a nuclear protein that is necessary for cellular proliferation and ribosomal RNA transcription[99]. It is present during all active phases of the cell cycle (G1, S, G2, and M), but is absent from resting cells (G0). The protein is predominantly localized in the peri-nucleolar region in the G 1 phase, in the later phases it is also detected throughout the nuclear interior, being predominantly localized in the nuclear matrix. In mitosis, it is present on all chromosomes[98]. Ki-67 is an excellent marker to determine the growth fraction of a given cell population. The fraction of Ki-67-positive tumor cells (the Ki-67 labeling index) is often correlated with the clinical course of various tumours like carcinomas of the prostate, brain and the breast. For these types of tumors, the prognostic values for survival and tumor recurrence have repeatedly been proven in uni- and multivariate analysis.

MIB-1 is a commonly used monoclonal antibody that detects the Ki-67 antigen. It is used in clinical applications to determine the Ki-67 labeling index. One of its primary advantages over the original Ki-67 antibody and the reason why it has essentially replaced the original antibody for clinical use is that it can be used on formalin-fixed paraffin-embedded sections, after heat-mediated antigen retrieval. Ki-67 labeling index is calculated by the percentage of tumours cells showing distinct brown staining of the nucleus with strong intratumoural heterogeneity. The other methods of detection of Ki-67 are by Western blot analysis and immunofluorescence.

The various other markers of proliferation include AgNOR staining, PCNA and Topoisomerase II. The novel markers being evaluated for identifying cell proliferation include Fen-1, MCM proteins (mini-chromosome maintenance), mitosin, polo – like kinase and claspin.

IMMUNOHISTOCHEMISTRY:

Albert Coons et al in 1941 first labeled antibodies directly with fluorescent isocyanate. Nakane and Pierce et al in 1966, introduced indirect labeling technique in which unlabeled antibody is followed by second antibody or substrate. Various stages of development of Immunohistochemistry include peroxidase – antiperoxidase method (1970), alkaline phosphatase labeling (1971), avidin biotin method (1977) and two layer dextrin polymer technique (1993)[92].

ANTIGEN RETRIEVAL:

Antigen retrieval can be done by the following different techniques to unmask the antigenic determinants of fixed tissue sections.

1. Proteolytic enzyme digestion
2. Microwave antigen retrieval
3. Pressure cooker antigen retrieval
4. Microwave and trypsin antigen retrieval

PROTEOLYTIC ENZYME DIGESTION:

Huank et al in 1976 introduced this technique to breakdown formalin cross linkages and to unmask the antigen determinants. The most commonly used enzymes include trypsin and proteinase[93]. The disadvantages include over digestion, under digestion and antigen destruction.

MICROWAVE ANTIGEN RETRIEVAL:

This is a new technique most commonly used in current practice. Microwave oven heating involves boiling formalin fixed paraffin sections in various buffers for rapid and uniform heating. Antibodies against Ki67 and MIB-1 work well after heat pretreatment in this method[92].

PRESSURE COOKER ANTIGEN RETRIEVAL:

Miller et al in 1995 compared and proved that pressure cooking method has fewer inconsistencies, less time consuming and can be used to retrieve large number of slides than in microwave method[94].

PITFALLS OF HEAT PRETREATMENT:

Drying of sections at any stage after heat pretreatment destroys antigenicity. Nuclear details are damaged in poorly fixed tissues. Fibers and fatty tissues tend to detach from slides while heating. Not all antigens are retrieved by heat pretreatment and also some antigens like PGP 9.5 show altered staining pattern.

DETECTION SYSTEMS:

After addition of specific antibodies to the antigens, next step is to visualize the antigen antibody reaction complex. The methods employed are direct and indirect methods.

In the direct method, primary antibody is directly conjugated with the label. Most commonly used labels are flouro-chrome, horse radish peroxidase and alkaline phosphatase. Indirect method is a two-step method in which labeled secondary antibody reacts with primary antibody bound to specific antigen. The use of peroxidase enzyme complex or avidin biotin complex further increases the sensitivity of immunohistochemical stains[92].

In 1993, Pluzek et al introduced enhanced polymer one step staining, in which large numbers of primary antibody and peroxidase enzymes are attached to dextran polymer back bone. This is the rapid and sensitive method[95].

Dextran polymer conjugate two step visualization system is based on dextran technology in Epos system. This method has greater sensitivity and is less time consuming.

MATERIALS AND METHODS

MATERIALS AND METHODS

This study is a retrospective descriptive study of gastric adenocarcinomas conducted in the Institute of Pathology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai during the period between January 2010 and December 2010.

A total of 9,541 cases were submitted to our department during the period January 2010 – December 2010 for histopathological examination. Among the 660 gastric specimens, 571 were endoscopic biopsies and 89 were gastrectomies. Among the 660 specimens, 297 were non neoplastic, 4 were benign and 359 were malignant tumours. A total of 275 endoscopic biopsy specimen and 84 gastrectomy specimens were reported to be malignant tumors. Out of the 89 gastrectomies, 78 gastrectomies were done to treat gastric carcinoma, 3 were done to treat GIST, 3 were done to treat Non – Hodgkin's lymphoma, 3 were done to treat giant bleeding benign ulcers, 1 was done to treat morbid obesity and 1 was done as revision gastrectomy to rule out stump carcinoma.

SOURCE OF DATA:

The gastric adenocarcinoma cases reported in gastrectomy specimens received in the Institute of Pathology, Madras Medical College between January 2010 to December 2010 from the Department of Surgery, Surgical Gastroenterology, Surgical Oncology and Geriatric surgery, Government

General Hospital. A total of 84 gastrectomy specimens (Subtotal, Total, Radical and Palliative gastrectomy) were received during this period.

Inclusion criteria

All the gastric carcinoma cases reported in gastrectomy specimens irrespective of the age and sex were included for the study.

Exclusion criteria

- Non neoplastic lesions and benign tumors of stomach.
- Gastric carcinomas reported in endoscopic biopsies.
- Gastrectomies performed for reasons other than treating carcinomas.

METHOD OF DATA COLLECTION:

Detailed history of the cases regarding age, sex, history, type of procedure, history of neo adjuvant therapy, details of gross characteristics and nodal status were obtained for all the 78 gastrectomy cases reported during the period of study from Surgical pathology records. Hematoxylin and Eosin stained 4 µ thick sections of the paraffin tissue blocks of gastrectomy specimens were reviewed. The following clinical and pathological parameters were evaluated: Age (<55 and >= 55), gender, tumour size (<5 and >=5cm), tumour location (Eso-cardiac, body, antrum, pangastric), macroscopic appearance (Borrmann Type I, Type II, Type III and Type IV). Carcinomas were classified as Intestinal and Diffuse based on the Lauren classification and into different

histological types (tubular, papillary, mucinous, signet ring cell and diffuse). Regarding the depth of invasion, the carcinomas were classified into 4 groups: T1 (invasion of mucosa and submucosa), T2 (invasion of muscularis propria and subserosa), T3 (invasion of serosa) and T4 (invasion of adjacent organs), and according to grade the carcinomas were divided into 3 groups: G1 (well differentiated), G2 (moderately differentiated) and G3 (poorly differentiated) according to the recommendations of the American Joint Committee on Cancer (2002). Lymph node metastasis was assessed and the patients were divided into 3 groups: N0 (No lymph node metastasis), N1 (metastasis in 1-6 nodes) and N2 (metastasis in 7 – 15 nodes). Carcinoma staging was done according to the standards of the American Joint Committee on Cancer (2002) and TNM classification of gastric carcinomas (Annexure – III). The tumours were further evaluated for the presence of necrosis, lymphocytic response, perineural invasion and lympho-vascular invasion by tumor and were graded as present or absent. 50 cases of gastric adenocarcinomas of varying grades were randomly selected from the total cases and their representative formalin fixed paraffin embedded tissue samples were subjected to immunohistochemistry for a panel of 2 markers – p53 and Ki-67.

IMMUNOHISTOCHEMICAL EVALUATION:

Immuohistochemical analysis of a panel of markers including p53 and Ki-67 were done in paraffin embedded tissue samples using Super-sensitive

polymer HRP system based on non-biotin polymeric technology. 4 µ thick sections from formalin fixed paraffin embedded tissue samples were transferred onto gelatin coated slides. Heat induced antigen retrieval was done. The antigen was bound with mouse monoclonal antibody (Biogenex) against p53 protein and Ki – 67 protein and then detected by the addition of secondary antibody conjugated with horse radish peroxidase-polymer and diaminobenzidine substrate. The step by step procedure of Immunohistochemistry is given in Annexure IV.

Antigen	Vendor	Species(clone)	Dilution	Positive control
P53	BIOGENEX	Mouse	Ready to use	Stomach
Ki - 67	BIOGENEX	Mouse	Ready to use	Stomach

INTERPRETATION & SCORING SYSTEM:

The immunohistochemically stained slides were analyzed for the presence of reaction, cellular localization, percentage of cells stained and intensity of reaction. Nuclear staining was assessed for both p53 and Ki 67. P53 immuno-reactivity was assessed as being positive when tumours exhibited intense nuclear staining and was categorized into 2 groups: Positive expression - (at least 10% positive tumour cell nuclei) and Negative expression - (less than 10% positive tumour cell nuclei)

A distinct nuclear immuno – reactivity for Ki -67 was considered positive. The Ki-67 labeling index was determined by observing 1000 cancer cell nuclei in areas of the section with highest labeling frequency. The Ki- 67 labeling index for the 50 tumours ranged from 3.9% to 75.3% with a mean labeling index of 25.4%. The mean Ki – 67 labeling index of 25.4% was chosen as the cut off point for separating the cases into 2 groups: High Ki – 67 labeling index (LI > 25.4%) and Low Ki – 67 labeling index (LI < 25.4%).

STATISTICAL ANALYSIS :

The statistical analysis was performed using statistical package for social science software version 11.5 which consisted computing the frequency counts and percentages for qualitative variables and mean for the quantitative variables. The expression of p53 and the Ki – 67 labeling index was correlated with clinico – pathological factors like age, gender, tumour site, tumour configuration, size, Lauren's type, histological types, histological grade, depth of infiltration, lymph node status, stage, lympho-vascular invasion, perineural invasion, lymphocytic response and necrosis using the Pearson's Chi –Square test. The expression of p53 and the Ki – 67 labeling index were also correlated with each other using the McNemar's test. T – test was used to detect the association between the mean Ki- 67 labeling index in the p53 positive and negative groups.

OBSERVATION AND RESULTS

OBSERVATION AND RESULTS

In the study period of 12 months from January2010 to December 2010, a total of 9,541 specimens were received in the Institute of Pathology, Madras Medical College for histological examination. Total numbers of gastric specimens received were 660, of these gastric tumors accounted for 359 with a percentage of 3.76 %. The total number of non- neoplastic, benign and malignant cases was 297, 4 and 359 respectively. Thus the distribution of non-neoplastic lesions was 45 %, of benign tumors were 0.6% and of malignant tumors were 54.39% among the gastric specimens.

Among the 660 gastric specimens, there were 89 gastrectomies. Of the 89 gastrectomies, 78 were done to treat gastric carcinoma, 3 were done to treat GIST, 3 were done to treat Non – Hodgkin's Lymphoma and the remaining 5 for non – neoplastic conditions. Thus the distribution of non-neoplastic lesions was 5.7% and malignant tumours was 94.3% among the gastrectomy specimens.

Gastric cancers had a peak incidence in the age group of 51-60 years. The youngest age of presentation of gastric cancer is at 23 years in this study. Among the 78 cases, 57 (73%) cases were reported in males and 21 (27%) cases were reported in females (Table 1 and Chart 1)

TABLE 1 AGE AND SEX WISE DISTRIBUTION OF GASTRIC CANCERS

AGE GROUP	NUMBER OF CANCERS		PERCENTAGE
	MALES	FEMALES	
21 – 30 years	0	2	2.5%
31 – 40 years	5	4	11.6%
41 – 50 years	11	6	21.8%
51 – 60 years	24	2	33.5%
61 – 70 years	11	7	23%
More than 70 years	6	0	7.6%
Total cases	57 (73%)	21 (27%)	100%

Among the 78 cases, 47 (60.3%) of cases involved the pyloro-antrum, 16 (20.5%) involved the body, 11 (14.1%) involved the eso-cardia and 4 (5.1%) cases were pan-gastric. (Table 2 and Chart 2)

TABLE 2 DISTRIBUTION OF SITE OF INVOLVEMENT IN GASTRIC CARCINOMA

SITE OF GASTRIC CANCER	NUMBER OF CASES	PERCENTAGE
Pyloro-antrum	47	60.3%
Body	16	20.5%
Eso-cardia	11	14.1%
Pan-gastric	4	5.1%
Total	78	100%

Based on the gross morphology, the gastric tumours were divided into 4 groups according to Borrmann's classification & the distribution is shown in Table 3 & Chart 3

TABLE 3 DISTRIBUTION OF GASTRIC CARCINOMA ACCORDING TO GROSS MORPHOLOGY

GROSS	NUMBER OF CASES	PERCENTAGE
Borrmann Type - I	12	15.3%
Borrmann Type - II	32	41%
Borrmann Type - III	25	32%
Borrmann Type - IV	8	10.2%
Early gastric cancer – Type III	1	1.2%
Total	78	100%

Among the study samples, 46 cases (58.9%) had tumor less than 5 cm in size and 32 cases (41%) were 5cm or more in size. (Table 4 & Chart 4)

TABLE 4 - DISTRIBUTION OF SIZE IN GASTRIC CARCINOMA

SIZE OF TUMOUR	NUMBER OF CASES	PERCENTAGE
<5 cm	46	58.9%
>=5 cm	32	41.1%
Total	78	100%

The distribution of histological subtypes of gastric carcinoma is shown in Table 5 & Chart 5.

TABLE 5 DISTRIBUTIONS OF HISTOLOGICAL SUBTYPES OF GASTRIC CANCERS

Histological subtypes	Number of cases	Percentage
Tubular carcinoma	42	53.9%
Papillary carcinoma	5	6.5%
Mucinous carcinoma	11	14.2%
Signet ring cell carcinoma	6	7.6%
Diffuse carcinoma	13	16.6%
Squamous cell carcinoma	1	1.2%
Total number of cases	78	100%

77 of the gastric adenocarcinomas were grouped into 2 according to Lauren's classification out of which 58 (75.4%) belonged to Intestinal type and 19 (24.6%) belonged to Diffuse type (Table 6 and Chart 6).

TABLE 6 DISTRIBUTION OF GASTRIC CANCER ACCORDING TO LAUREN'S CLASSIFICATION

LAUREN'S TYPE	NUMBER OF CASES	PERCENTAGE
Intestinal type	58	75.4%
Diffuse type	19	24.6%
Total	77	100%

The gastric carcinomas were graded according to AJCC recommendation and were divided into 3 groups, out of which 11 cases (14.2%) were well

differentiated (G1), 38 cases (48.7%) were moderately differentiated (G2) and 29 cases (37.1%) were in poorly differentiated (G3). (Table 7 & Chart 7)

TABLE 7 DISTRIBUTION OF HISTOLOGICAL GRADE IN GASTRIC CARCINOMAS

GRADE	NUMBER OF CASES	PERCENTAGE
G1	11	14.2%
G2	38	48.7%
G3	29	37.1%
TOTAL	78	100%

In this study, 1 case (1.2%) showed invasion upto the submucosa (T1), 36 cases (46.2%) showed infiltration into the muscularis propria or subserosa (T2), 36 cases (46.2%) showed infiltration into the serosa and 5 cases (6.4%) showed infiltration of adjacent organs (T4) (Table 8 and Chart 8).

TABLE 8 DISTRIBUTION OF GASTRIC CARCINOMAS ACCORDING TO DEPTH OF INVASION

DEPTH OF INVASION	NUMBER OF CASES	PERCENTAGE
T1	1	1.2%
T2	36	46.2%
T3	36	46.2%
T4	5	6.4%
Total	78	100%

This study showed that 39 cases (50%) had up to 6 nodes with metastatic carcinomatous deposit (N1), 5 cases (6.4%) had 7 to 15 involved nodes (N2) while 34 cases (43.6%) had no node involvement (N0). (Table 9 & Chart 9)

TABLE 9 DISTRIBUTION OF LYMPH NODE METASTASIS IN GASTRIC CANCERS

Lymph node status	Number of cases	Percentage
N0	34	43.6%
N1	39	50%
N2	5	6.4%
Total	78	100%

In the present study, 17 cases (21.8%) belonged to stage I, 35 cases (44.9%) belonged to stage II, 21 cases (26.9%) belonged to stage III and 5 cases (6.4%) belonged to stage IV. (Table 10 and Chart 10)

TABLE 10 DISTRIBUTION OF GASTRIC CARCINOMAS ACCORDING TO STAGE

STAGE	NUMBER OF CASES	PERCENTAGE
I	17	21.8%
II	35	44.9%
III	21	26.9%
IV	5	6.4%
Total	78	100%

In this study, among the 78 cases, 52 cases (66.6%) had lymphatic invasion as against 26 cases (33.4%) without lymphatic invasion. 15 cases (19.3%) showed vascular invasion while 63 cases (80.7%) cases had no vascular invasion, 17.9 % of the cases had perineural infiltration, 85.8% of the cases had lymphocytic infiltration , 23.1% of the cases had necrosis. (Table 11 & Chart 11)

TABLE 11 DISTRIBUTION OF OTHER PROGNOSTIC FACTORS IN GASTRIC CARCINOMA

Patient characteristics	Present	Absent	Total
Lymphatic invasion	52 (66.6%)	26 (33.4%)	78 (100%)
Vascular invasion	15 (19.3%)	63 (80.7%)	78 (100%)
Perineural infiltration	14 (17.9%)	64 (82.1%)	78 (100%)
Lymphocytic infiltration	67 (85.8%)	11 (14.2%)	78 (100%)
Necrosis	18 (23%)	60 (77%)	78 (100%)

RESULTS OF IMMUNOHISTOCHEMICAL STUDIES

Of the total 78 cases, 50 cases of varying grade and stage were selected in a random manner and subjected to immunohistochemical analysis with a panel of 2 markers – p53 and Ki-67.

Of the 50 cases, there were 39 males (78%) and 11 females (22%). The ages ranged between 28 and 75 with a mean of 55.04. There were 18 cases (36%) below 55 years of age and 32 cases (64%) more than 55 years. The

tumour was located in the pyloro – antrum in 27 cases (54%), body in 12 cases (24%), eso-cardia in 7 cases (14%) and were pan-gastric in 4 cases (8%). 10 cases (20%) belonged to Borrmann Type I, 20 cases (40%) belonged to Type II, 14 cases (28%) belonged to Type III and 6 cases (12%) belonged to type IV. The tumours ranged in size from 2 to 12 cm with an average of 5.72.

There were 27 cases (54%) with tumour size <5 cm and 23 cases (46%) with size >5cm. 31 cases (62%) were of the tubular type, 4 cases (8%) were of the papillary type, 6 cases (12%) were mucinous carcinomas, 3 cases (6%) were of the signet ring cell type and 6 cases (12%) were of the diffuse type. 41 cases (82%) belonged to Lauren's Intestinal type and 9 cases (18%) belonged to the Diffuse type.

Among the final study group, 9 (18%) cases were of G1, 25 (50%) cases were of G2 and 16 (32%) cases were of G3. 23 (46%) cases belonged to T2, 24 (48%) cases belonged to T3 and 3 cases (6%) belonged to T4. Of the 50 cases, 35(70%) showed lymphatic invasion, 13 cases (26%) showed vascular invasion, 10 cases (20%) showed perineural invasion, 46 (92%) cases showed lymphocytic response and 17(34%) showed necrosis. Nodal metastasis was present in 1-6 nodes (N1) in 23 cases (46%), 7-15 nodes (N2) in 5 cases (10%) and absent in 22 (44%) cases. 12 (24%) cases belonged to stage I, 19 (38%) cases belonged to stage II, 16 cases (32%) belonged to stage III and 3 cases (6%) belonged to stage IV. (Table 12)

TABLE 12 - DISTRIBUTION OF GASTRIC CARCINOMA AMONG THE VARIOUS CLINICOPATHOLOGICAL GROUPS FOR THE IHC STUDY (50 CASES)

Clinico-pathological factor		No. of cases
Age	<55	18 (36%)
	>55	32 (64%)
Sex	Males	39 (78%)
	Females	11 (22%)
Site	Pyloro -antrum	27 (54%)
	Body	12 (24%)
	Eso- cardia	7 (14%)
	Pan - gastric	4 (8%)
Borrmann	I	10 (20%)
	II	20 (40%)
	III	14 (28%)
	IV	6 (12%)
Size	<5cm	27 (54%)
	>5cm	23 (46%)
Histological type	Tubular	31 (62%)
	Papillary	4 (8%)
	Mucinous	6 (12%)
	Signet ring cell	3 (6%)
	Diffuse	6 (12%)
Lauren	Intestinal	41 (82%)
	Diffuse	9 (18%)
Grade	G1	9 (18%)
	G2	25 (50%)
	G3	16 (32%)
Depth	T2	23 (46%)
	T3	24 (48%)
	T4	3 (6%)
Lymphatic invasion	P/A	35 (70%) / 15 (30%)
Vascular invasion	P/A	13 (26%) / 37 (74%)
Perineural invasion	P/A	10 (20%) / 40 (80%)
Lymphocytic response	P/A	46 (92%) / 4 (8%)
Necrosis	P/A	17(34%) / 33 (66%)
Lymph nodes	N0	22 (44%)
	N1	23 (46%)
	N2	5 (10%)
Stage	I	12(24%)
	II	19 (38%)
	III	16 (32%)
	IV	3 (6%)

In this study, 32 cases (64%) expressed positive reaction for p53 and 18 cases (36%) were p53 negative. With the mean Ki-67 as 25.4%, the cases were divided into two groups – High Ki-67 labeling index which was present in 19 cases (38%) and Low Ki-67 labeling index which was present in 31 cases (62%). (Table 13 & Chart 12)

TABLE 13 - DISTRIBUTION OF p53 EXPRESSION AND Ki -67 LI IN GASTRIC CARCINOMA

IHC PARAMETER	P53		Ki-67 LI	
RESULT	POSITIVE	NEGATIVE	HIGH	LOW
	32 (64%)	18 (36%)	19 (38%)	31(62%)
TOTAL(%)	50 (100%)		50 (100%)	

CORRELATION OF p53 WITH VARIOUS CLINICOPATHOLOGICAL FACTORS

p53 positivity was noted in 50% patients with age less than 55 and in 71.9% patients with age more than 55. (Table 14 & Chart 13)

TABLE 14 CORRELATION OF AGE WITH p53 EXPRESSION

Age (yrs)	p53 positive (%)	p53 negative (%)	Total	Pearson chi square test
<55	9(50%)	9(50%)	18(100%)	P=0.215
>55	23(71.9%)	9(28.1%)	32 (100%)	
Total	32 (64%)	18 (36%)	50 (100%)	

p53 positivity was obtained in 66.7% of men and 54.5% of women, noting a slight predominance in males. (Table 15 & Chart 14)

TABLE 15 CORRELATION OF GENDER WITH p53 EXPRESSION

Gender	p53 positive (%)	p53 negative (%)	Total	Pearson chi square test
Male	26(66.7%)	13(33.3%)	39(100%)	P=0.701
Female	6(54.5%)	5(45.5%)	11(100%)	
Total	32 (64%)	18 (36%)	50 (100%)	

In the present study, p53 positivity was observed in 74.1% of tumours of the pyloro – antrum, 66.7% of tumours of the body, 14.3% of tumours of the eso – cardia and 75% of pan – gastric tumours. The association with respect to site was found to be significant with increased expression seen in tumours of the pyloro – antrum and in pan – gastric tumours. (Table 16 and Chart15)

TABLE 16 CORRELATION OF TUMOUR SITE WITH p53 EXPRESSION

Site	p53 positive (%)	p53 negative (%)	Total	Pearson chi square test
P - antrum	20(74.1%)	7(25.9%)	27(100%)	P=0.030
Body	8(66.7%)	4(33.3%)	12(100%)	
Eso – cardia	1(14.3%)	6 (85.7%)	7 (100%)	
Pan - gastric	3 (75%)	1 (25%)	4 (100%)	
Total	32 (64%)	18 (36%)	50 (100%)	

Among the various gross types, p53 positivity was noted in 6 cases (60%) of Borrmann type I, 11 cases (55%) of Borrmann type II, 10 cases (71.4%) of

Borrmann type III and 5 cases (83.3%) of Borrmann type IV. (Table 17 and Chart16)

TABLE 17 CORRELATION OF GROSS TYPE WITH p53 EXPRESSION

Gross	p53 positive (%)	p53 negative (%)	Total	Pearson chi square test
Type I	6(60%)	4(40%)	10(100%)	P=0.555
Type II	11(55%)	9(45%)	20(100%)	
Type III	10(71.4%)	4 (28.6%)	14 (100%)	
Type IV	5 (83.3%)	1 (16.7%)	6 (100%)	
Total	32 (64%)	18 (36%)	50 (100%)	

In the present study, p53 positivity was noted in an increased frequency (70.4%) in cases with tumour size <5cm compared to the 56.5% of cases with size >=5cm. (Table 18 and Chart 17)

TABLE 18 CORRELATION OF TUMOUR SIZE WITH p53 EXPRESSION

Size	p53 positive (%)	p53 negative (%)	Total	Pearson chi square test
<5 cm	19(70.4%)	8(29.6%)	27(100%)	P=0.471
>=5 cm	13(56.5%)	10(43.5%)	23(100%)	
Total	32 (64%)	18 (36%)	50 (100%)	

Among histological forms, 64.5% of tubular carcinomas, 25% of papillary carcinoma, 33.3% of signet ring cell carcinomas and 66.7% of diffuse carcinomas showed p53 positivity. In this study, 100% of mucinous carcinomas showed p53 positivity. (Table 19 and Chart 18)

TABLE 19 CORRELATION OF HISTOLOGICAL TYPE WITH p53 EXPRESSION

His.type	p53 positive (%)	p53 negative (%)	Total	Pearson chi square test
Tubular	20(64.5%)	11(35.5%)	31(100%)	P=0.123
Papillary	1(25%)	3(75%)	4(100%)	
Mucinous	6 (100%)	0 (0%)	6 (100%)	
Signet	1 (33.3%)	2 (66.7%)	3 (100%)	
Diffuse	4 (66.7%)	2 (33.3%)	6 (100%)	
Total	32 (64%)	18 (36%)	50(100%)	

When Lauren's classification was taken into account, a greater frequency of p53 positivation with Intestinal type cancers (65.8%) in comparison with diffuse type carcinomas (55.6%) was observed. (Table 20 and Chart 19)

TABLE 20 CORRELATION OF LAUREN'S HISTOLOGICAL TYPE WITH p53 EXPRESSION

Lauren type	p53 positive (%)	p53 negative (%)	Total	Pearson chi square test
Intestinal	27(65.8%)	14(34.2%)	41(100%)	P=0.560
Diffuse	5(55.6%)	4(44.4%)	9(100%)	
Total	32 (64%)	18 (36%)	50 (100%)	

An increasing percentage of cases showing p53 positivity with increasing tumour grade was observed. 44.4% of well differentiated tumours (G1), 64% of

moderately differentiated tumours (G2) and 75% of poorly differentiated tumours (G3) showing positivity for p53 was observed. (Table 21 and Chart 20)

TABLE 21 CORRELATION OF TUMOUR GRADE WITH p53 EXPRESSION

Grade	p53 positive (%)	p53 negative (%)	Total	Pearson chi square test
G1	4(44.4%)	5(55.6%)	9(100%)	P=0.311
G2	16(64%)	9(36%)	25(100%)	
G3	12 (75%)	4 (25%)	16 (100%)	
Total	32 (64%)	18 (36%)	50 (100%)	

According to the T stage, a progressive increase in the number of p53 positive cases from T2 to T3 was noted. p53 positivity was identified in 52.2% of T2 carcinomas, 75% of T3 carcinomas and 66.7% of T4 carcinomas.(Table 22 and Chart 21)

TABLE 22 CORRELATION OF T STAGE WITH p53 EXPRESSION

T stage	p53 positive (%)	p53 negative (%)	Total	Pearson chi square test
T2	12(52.2%)	11(47.8%)	23(100%)	P=0.264
T3	18(75%)	6(25%)	24(100%)	
T4	2 (66.7%)	1 (33.3%)	3 (100%)	
Total	32 (64%)	18 (36%)	50 (100%)	

In this study, a progressive increase in the percentage of p53 positive cases with an increased N stage was observed. p53 positivity was noticed in

59.1 % of N0 cases, 60.9% of N1 cases and 100% of N2 cases.(Table 23 and Chart 22)

TABLE 23 CORRELATION OF N STAGE WITH p53 EXPRESSION

N stage	p53 positive (%)	p53 negative (%)	Total	Pearson chi square test
N0	13(59.1%)	9(40.9%)	22(100%)	P=0.208
N1	14(60.9%)	9(39.1%)	23(100%)	
N2	5 (100%)	0 (0%)	5 (100%)	
Total	32 (64%)	18 (36%)	50 (100%)	

p53 positivity was noticed in 50% of stage I cases, 63.2% of stage II cases, 75% of Stage III cases but only 66.7 % of Stage IV cases. (Table 24 and Chart 23)

TABLE 24 CORRELATION OF TNM STAGE WITH p53 EXPRESSION

Stage	p53 positive (%)	p53 negative (%)	Total	Pearson chi square test
I	6(50%)	6(50%)	12(100%)	P=0.505
II	12(63.2%)	7(36.8%)	19(100%)	
III	12 (75%)	4 (25%)	16 (100%)	
IV	2 (66.7%)	1 (33.3%)	3 (100%)	
Total	32 (64%)	18 (36%)	50 (100%)	

In this study, a significant increase in the number of p53 positive cases in the presence of lymphatic invasion was observed. A higher percentage of p53 positive cases were found in tumours having perineural infiltration, vascular invasion, lymphocytic response and necrosis. p53 positivity was noted in 74.3%

cases with lymphatic invasion, 76.9% cases with vascular invasion, 80% cases with perineural infiltration, 63% cases with lymphocytic response and 70.6% cases with necrosis. (Table 25)

TABLE 25 CORRELATION OF p53 WITH PROGNOSTIC PARAMETERS

Patient characteristics		p53		Pearson chi-square test
		Positive	Negative	
Perineural infiltration	Present	8(80%)	2(20%)	P=0.239
	Absent	24(60%)	16(40%)	
Lymphatic invasion	Present	26(74.3%)	9(25.7%)	P=0.046
	Absent	6(40%)	9(60%)	
Vascular invasion	Present	10(76.9%)	3(23.1%)	P=0.428
	Absent	22(59.5%)	15(40.5%)	
Lymphocytic infiltration	Present	29(63%)	17(37%)	P=0.633
	Absent	3(75%)	1(25%)	
Necrosis	Present	12(70.6%)	5(29.4%)	P=0.700
	Absent	20(60.6%)	13(39.4%)	

The present study showed that there was statistically significant association between p53 expression and tumour location and lymphatic invasion. p53 over-expression was seen to increase with increasing age, grade, depth of infiltration, nodal stage and TNM stage. But when subjected to statistical analysis this association was not found to be significant. There was a

slight predominance in males and intestinal type tumours. Increased p53 expression was noted in Borrmann type III and type IV tumours.100% of mucinous carcinomas showed p53 positivity .

CORRELATION OF Ki-67 LABELING INDEX WITH VARIOUS CLINICO – PATHOLOGICAL PARAMETERS

With a mean Ki-67 labeling index of 25.4%, the study group was divided into 2 groups. 38% cases showed high Ki-67 labeling index and 62% cases showed low Ki- 67 labeling index.

High Ki-67 LI was noted in 38.9% patients with age less than 55 and in 37.5% patients with age more than 55. (Table 26 & Chart 13)

TABLE 26 CORRELATION OF AGE WITH Ki -67 LI

Age (yrs)	High LI (%)	Low LI (%)	Total	Pearson chi square test
<55	7(38.9%)	11(61.1%)	18(100%)	P=0.923
>55	12(37.5%)	20(62.5%)	32 (100%)	
Total	19 (38%)	31 (62%)	50 (100%)	

High Ki-67 LI was noted in 38.5% of men and 36.4% of women, noting a slight predominance in males. (Table 27 & Chart 14)

TABLE 27 CORRELATION OF GENDER WITH Ki-67 LI

Gender	High LI (%)	Low LI (%)	Total	Pearson chi square test
Male	15(38.5%)	24(61.5%)	39(100%)	P=0.899
Female	4(36.4%)	7(63.6%)	11(100%)	
Total	19 (38%)	31 (62%)	50 (100%)	

In the present study, high Ki -67 LI was observed in 44.4% of tumours of the pyloro – antrum, 41.7% of tumours of the body, 28.6% of tumours of the eso – cardia and 0% of pan – gastric tumours. (Table 28 and Chart 15)

TABLE 28 CORRELATION OF TUMOUR SITE WITH Ki-67 LI

Site	High LI (%)	Low LI (%)	Total	Pearson chi square test
P - antrum	12(44.4%)	15(55.6%)	27(100%)	P=0.353
Body	5(41.7%)	7(58.3%)	12(100%)	
Eso – cardia	2(28.6%)	5 (71.4%)	7 (100%)	
Pan - gastric	0 (0%)	4 (100%)	4 (100%)	
Total	19 (38%)	31 (62%)	50 (100%)	

Among the various gross types, high Ki-67 LI was noted in 4 cases (40%) of Borrmann type I, 8 cases (40%) of Bormann type II, 6 cases (42.8%) of Borrmann type III and 1 case (16.7%) of Borrmann type IV. (Table 29 and Chart 16)

TABLE 29 CORRELATION OF GROSS TYPE WITH Ki – 67 LI

Gross	High LI (%)	Low LI (%)	Total	Pearson chi square test
Type I	4(40%)	6(60%)	10(100%)	P=0.513
Type II	8(40%)	12(60%)	20(100%)	
Type III	6(42.8%)	8 (57.2%)	14 (100%)	
Type IV	1(16.7%)	5 (83.3%)	6 (100%)	
Total	19 (38%)	31 (62%)	50 (100%)	

In the present study, high Ki-67 LI was noted in 48.1% of cases with tumour size <5cm compared to the 26.1% of cases with size >=5cm. (Table 30 and Chart 17)

TABLE 30 CORRELATION OF TUMOUR SIZE WITH Ki-67 LI

Size	High LI (%)	Low LI (%)	Total	Pearson chi square test
<5 cm	13(48.1%)	14(51.9%)	27(100%)	P=0.190
>=5 cm	6(26.1%)	17(73.9%)	23(100%)	
Total	19 (38%)	31 (62%)	50 (100%)	

Among histological forms, 35.5% of tubular carcinomas, 25% of papillary carcinomas, 66.7% of mucinous carcinoma and signet ring cell carcinomas and 16.7% of diffuse carcinomas showed high Ki-67 LI. In this study, higher percentage of cases with high Ki-67 LI was noted in mucinous and signet ring cell carcinomas and higher percentage of cases with low Ki-67 LI was found in tubular and papillary carcinomas. (Table 31 and Chart 18)

TABLE 31 CORRELATION OF HISTOLOGICAL TYPE WITH Ki-67 LI

His.type	High LI (%)	Low LI (%)	Total	Pearson chi square test
Tubular	11(35.5%)	20(64.5%)	31(100%)	P=0.123
Papillary	1(25%)	3(75%)	4(100%)	
Mucinous	4 (66.7%)	2 (33.3%)	6 (100%)	
Signet	2 (66.7%)	1 (33.3%)	3 (100%)	
Diffuse	1 (16.7%)	5 (83.3%)	6 (100%)	
Total	19(38%)	31 (62%)	50(100%)	

According with Lauren's classification, a slightly increased frequency of intestinal type cancers (39%) showing high Ki-67 LI in comparison with diffuse type carcinomas (33.3%) was noted. (Table 32 and Chart 19)

TABLE 32 CORRELATION OF LAUREN'S HISTOLOGICAL TYPE WITH Ki-67 LI

Lauren type	High LI (%)	Low LI (%)	Total	Pearson chi square test
Intestinal	16(39%)	25(61%)	41(100%)	P=0.614
Diffuse	3(33.3%)	6(66.7%)	9(100%)	
Total	19 (38%)	31 (62%)	50 (100%)	

It was observed that there was an increasing percentage of cases showing high Ki-67 LI as the grade increased. 44.4% of moderately differentiated tumours (G2) and 43.8 % of poorly differentiated tumours (G3) showed high Ki-67 LI. A significant percentage (88.9%) of well differentiated tumours (G1) showed low Ki -67 LI. (Table 33 and Chart 20)

TABLE 33 CORRELATION OF TUMOUR GRADE WITH Ki- 67 LI

Grade	High LI (%)	Low LI (%)	Total	Pearson chi square test
G1	1(11.1%)	8(88.9%)	9(100%)	P=0.186
G2	11(44%)	14(56%)	25(100%)	
G3	7 (43.8%)	9 (56.2%)	16 (100%)	
Total	19(38%)	31 (62%)	50 (100%)	

According to the T stage, a progressive increase in the number of cases showing high Ki-67 LI from T2 to T3 was noted. The study identified high Ki-67 LI in 30.4% of T2 tumours, 45.8% of T3 tumours and 33.3% of T4 tumours. (Table 34 and Chart 21).

TABLE 34 CORRELATION OF T STAGE WITH Ki-67 LI

T stage	High LI (%)	Low LI (%)	Total	Pearson chi square test
T2	7(30.4%)	16(69.6%)	23(100%)	P=0.546
T3	11(45.8%)	13(54.2%)	24(100%)	
T4	1 (33.3%)	2 (66.7%)	3 (100%)	
Total	19 (38%)	31 (62%)	50 (100%)	

A progressive increase in the percentage of cases with high Ki-67 LI with an increased N stage was observed. High LI was noticed in 34.8% of N1 cases and 60% of N2 cases. 63.6% of cases with no lymph node metastasis showed low LI (Table 35 and Chart 22).

TABLE 35 CORRELATION OF N STAGE WITH Ki-67 LI

N stage	High LI (%)	Low LI (%)	Total	Pearson chi square test
N0	8(36.4%)	14(63.6%)	22(100%)	P=0.562
N1	8(34.8%)	15(65.2%)	23(100%)	
N2	3 (60%)	2 (40%)	5 (100%)	
Total	19 (38%)	31 (62%)	50 (100%)	

High LI was observed in 33.3% of stage I cases, 26.3% of stage II cases, 56.2% of Stage III cases but only 33.3 % in Stage IV cases (Table 36 and Chart 23).

TABLE 36 CORRELATION OF TNM STAGE WITH Ki-67 LI

Stage	High LI (%)	Low LI (%)	Total	Pearson chi square test
I	4(33.3%)	8(66.7%)	12(100%)	P=0.320
II	5(26.3%)	14(73.7%)	19(100%)	
III	9 (56.2%)	7 (43.8%)	16 (100%)	
IV	1 (33.3%)	2 (66.7%)	3 (100%)	
Total	19 (38%)	31 (62%)	50 (100%)	

In this study, high Ki67 Li was found in 42.9% of cases with lymphatic invasion, 69.2% of cases with vascular invasion, 70% of cases with perineural infiltration, 39.1% of cases with lymphocytic infiltration and 41.2% of cases with necrosis. The association between high LI and vascular invasion and perineural infiltration was found to be statistically significant. A higher

percentage of cases with high LI were found in tumours having lymphatic invasion, lymphocytic response and necrosis. (Table 37)

TABLE 37 CORRELATION OF Ki-67 LI WITH PROGNOSTIC PARAMETERS

Patient characteristics		Ki-67 LI		Pearson chi-square test
		High	Low	
Perineural infiltration	Present	7(70%)	3(30%)	P=0.049
	Absent	12(30%)	28(70%)	
Lymphatic invasion	Present	15(42.9%)	20(57.1%)	P=0.445
	Absent	4(26.7%)	11(73.3%)	
Vascular invasion	Present	9(69.2%)	4(30.8%)	P=0.018
	Absent	10(27%)	27(73%)	
Lymphocytic infiltration	Present	18(39.1%)	28(60.9%)	P=0.983
	Absent	1(25%)	3(75%)	
Necrosis	Present	7(41.2%)	10(58.8%)	P=0.980
	Absent	12 (36.4%)	21(63.6%)	

The present study showed increased Ki-67 LI in Borrmann type III tumours, mucinous, signet ring cell and intestinal type carcinomas. A progressive increase in the number of cases showing high Ki-67 LI was noticed with increasing grade, depth of infiltration and nodal stage.

In this study, when high grade tumours were compared, it was found that the cases with nodal metastasis were positive for p53 immunoreaction but had low Ki-67 LI and the cases with no nodal involvement were p53 negative with high Ki-67 LI. It was also observed that a few low grade tumours with no nodal metastasis were p53 positive and had a low Ki-67 LI.

CORRELATION OF p53 EXPRESSION WITH KI-67 LABELING INDEX

High Ki-67 labeling index was found in 43.8% of patients who showed p53 positivity and low Ki-67 LI was found in 72.2% of patients with p53 negativity. Using the McNemar's test, the p value was found to be 0.011 which indicated no relation between p53 expression and Ki-67 LI. They were found to be independent prognostic variables with different outcomes for the various clinico-pathological factors. (Table 38 and Chart 24)

TABLE 38 CORRELATION OF KI-67 LI WITH P53 EXPRESSION

p53	Ki – 67 labeling index		Total	McNemar's Test
	High	Low		
Positive (%)	14(43.8%)	18(56.2%)	32 (100%)	P=0.011
Negative (%)	5 (27.8%)	13 (72.2%)	18 (100%)	

The mean Ki-67 LI value of p53 positive tumours was 29.362 and was significantly higher than that of p53 negative tumours. (Table 39 and Chart 25)

TABLE 39 MEAN Ki 67 LI IN p53 POSITIVE AND NEGATIVE GASTRIC TUMOURS

p53	N	Mean Ki-67 LI	t-test for equality of means
Positive	32	29.362	P = 0.038
Negative	18	18.361	

GASTRIC ADENOCARCINOMA – BORRMANN TYPE I

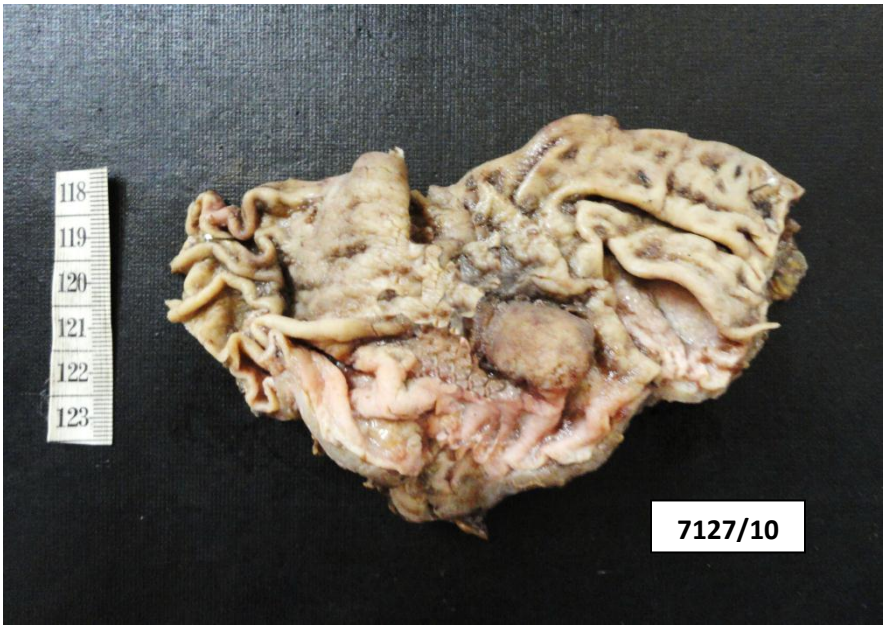


FIGURE 3 : Gastric adenocarcinoma (IT) – Nodular

GASTRIC ADENOCARCINOMA – BORRMANN TYPE I

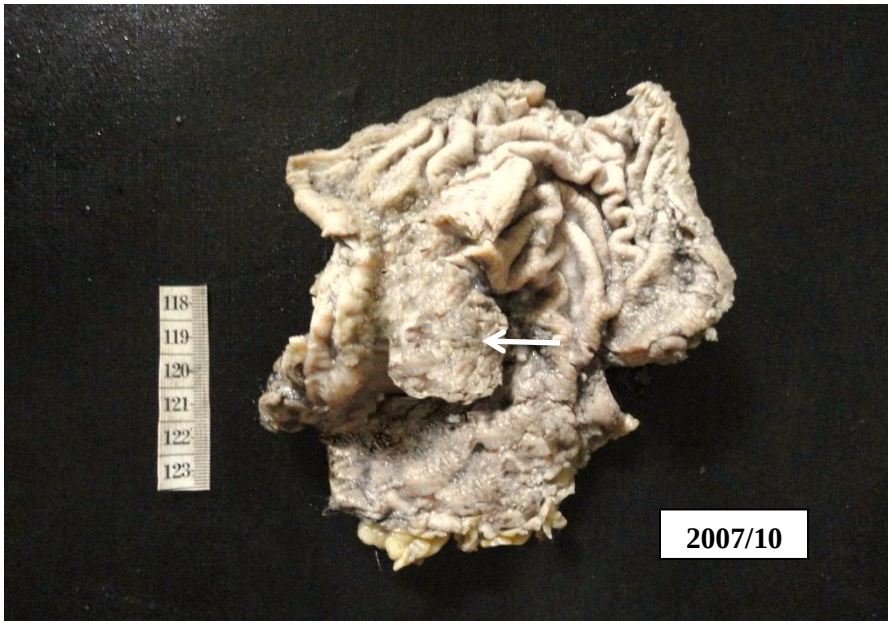


FIGURE 4 : Gastric adenocarcinoma (DT) – Nodular

GASTRIC ADENOCARCINOMA – BORRMANN TYPE II

FIGURE 5: Gastric adenocarcinoma (IT) – Localised ulcer

GASTRIC ADENOCARCINOMA –BORRMANN TYPE II

FIGURE 6: Gastric adenocarcinoma (DT) – Fungating

GASTRIC ADENOCARCINOMA – BORRMANN TYPE III

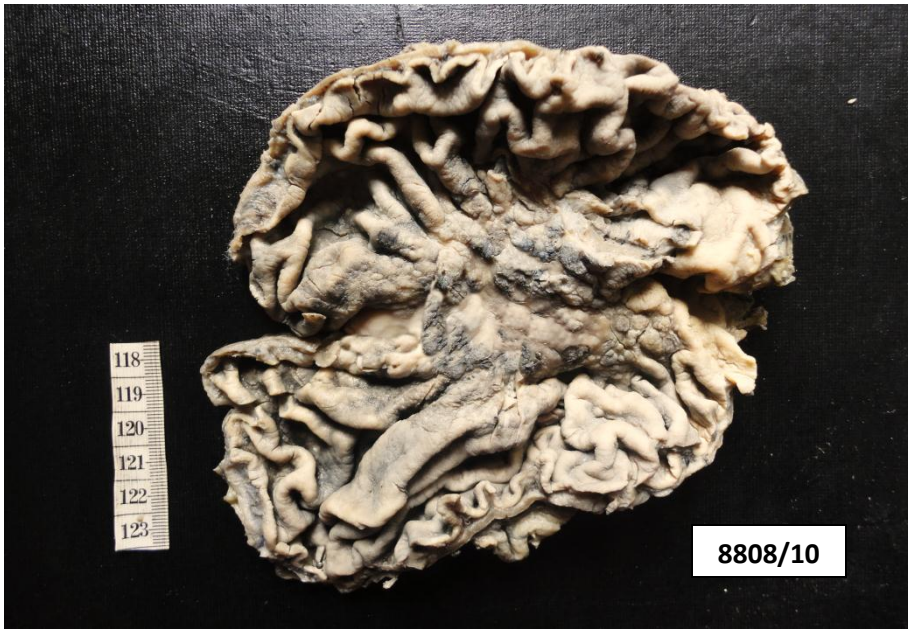


FIGURE 7 : Gastric adenocarcinoma (IT) – Ulcerative, infiltrative

GASTRIC ADENOCARCINOMA – BORRMANN TYPE III

FIGURE 8 : Gastric adenocarcinoma (DT) – Ulcerative, infiltrative

GASTRIC ADENOCARCINOMA – BORRMANN TYPE IV

FIGURE 9 : Gastric adenocarcinoma (DT) – Linitis Plastica

MUCINOUS CARCINOMA

FIGURE 10 : Diffuse glistening gelatinous growth

PAPILLARY CARCINOMA

FIGURE 11 : Ulcerative growth with raised edges

KRUKENBERG TUMOUR

FIGURE 12 : Solid grey – tan ovarian mass with surface bosselation

GASTRIC ADENOCARCINOMA – INTESTINAL TYPE – GRADE I

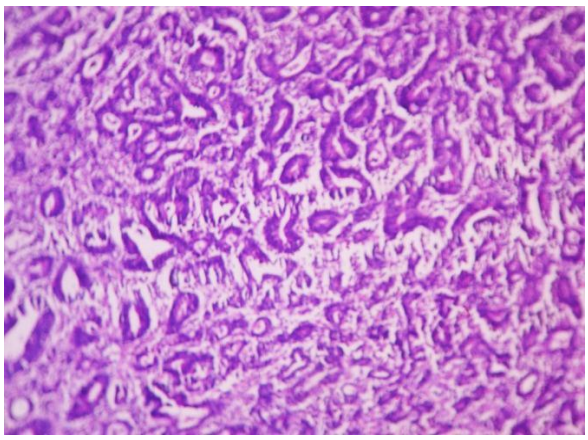


FIGURE 13: IT – Well differentiated with well formed glands (100X) HPE – 938/10

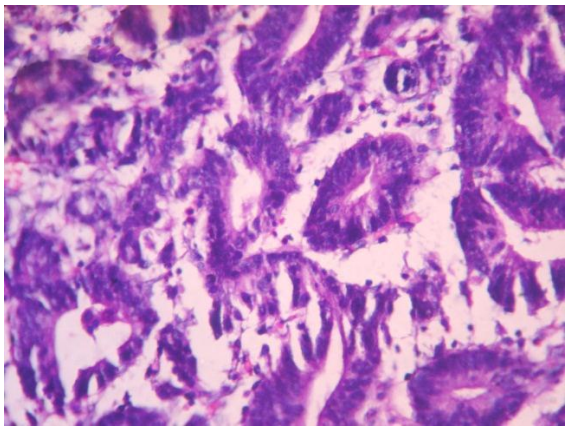


FIGURE 14 : Malignant epithelial cells in gland formation with nuclear stratification and pleomorphism. (400X) HPE – 938/10

GASTRIC ADENOCARCINOMA – INTESTINAL TYPE – GRADE II

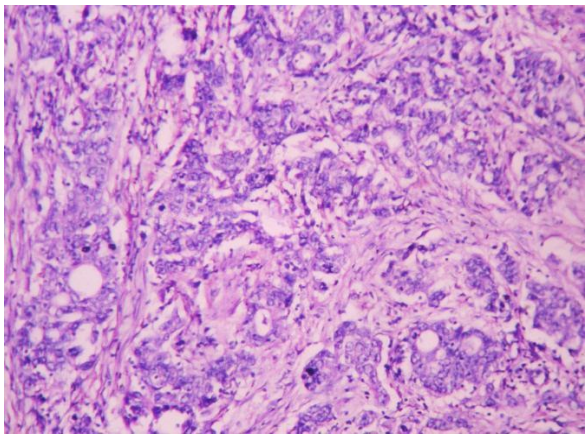


FIGURE 15 : IT – Moderately differentiated with sheets and glandular formation (100X) HPE – 3584/10

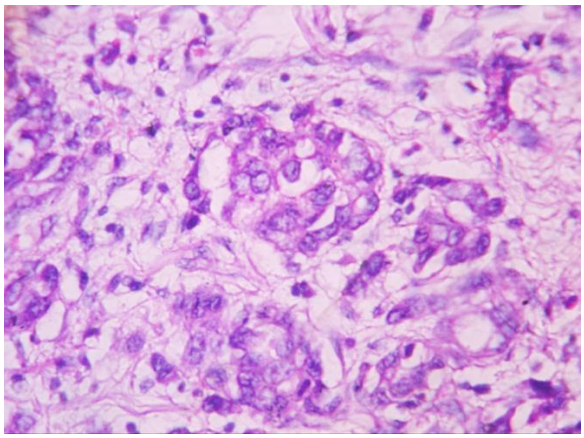


FIGURE 16 : Malignant epithelial cells with nuclear pleomorphism and intracellular mucin. (400X) HPE – 3584/10

GASTRIC ADENOCARCINOMA – INTESTINAL TYPE – GRADE III

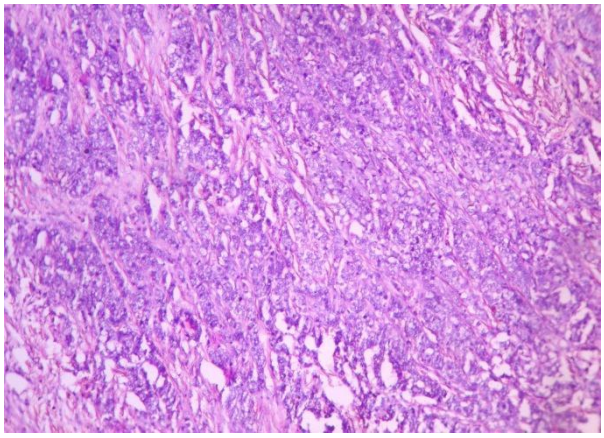


FIGURE 17 : IT – Poorly differentiated with malignant epithelial cells arranged in sheets. (100X) HPE - 4821/10

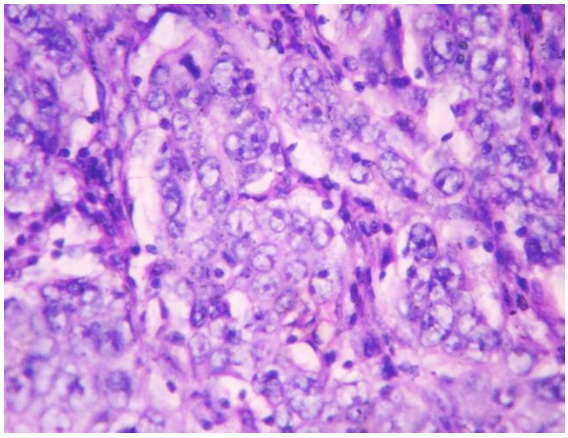


FIGURE 18 : Malignant epithelial cells in sheets with nuclear pleomorphism and prominent nucleoli (400X) HPE – 4821/10

GASTRIC ADENOCARCINOMA – DIFFUSE TYPE

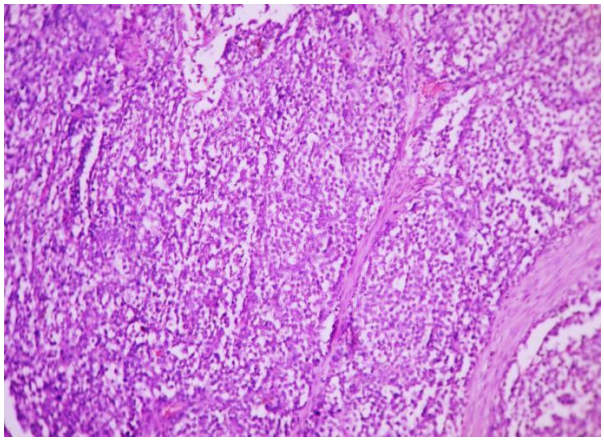


FIGURE 19 : Poorly cohesive cells diffusely infiltrating the gastric wall. (100X) HPE – 2456/10

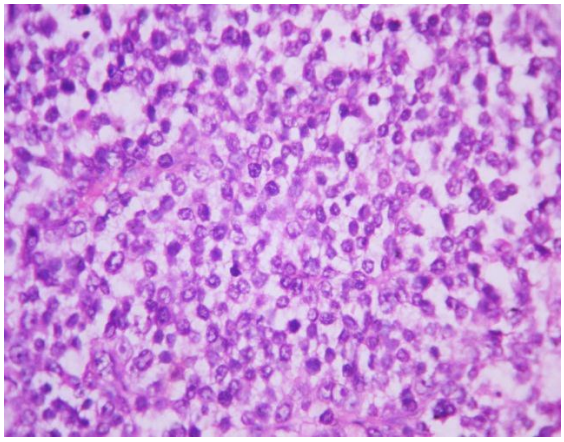


FIGURE 20 : Small and round poorly cohesive cells (400X) HPE – 2456/10

TUBULAR ADENOCARCINOMA

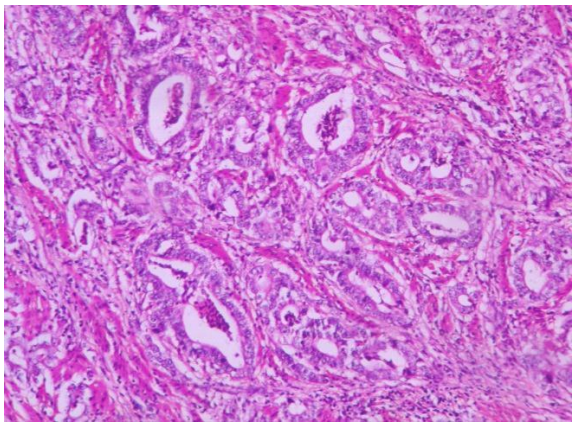


FIGURE 21 : Numerous dilated, slit like and Irregularly branching tubules of varying size (100X) HPE – 2678/10

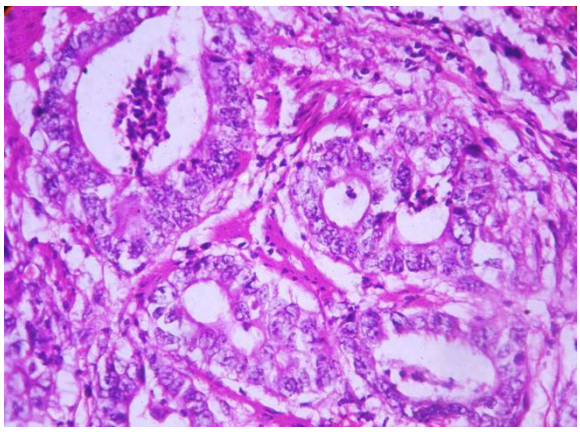


FIGURE 22 : Tubules lined by cuboidal to columnar cells with cytological atypia (400X) HPE – 2678/10

PAPILLARY ADENOCARCINOMA

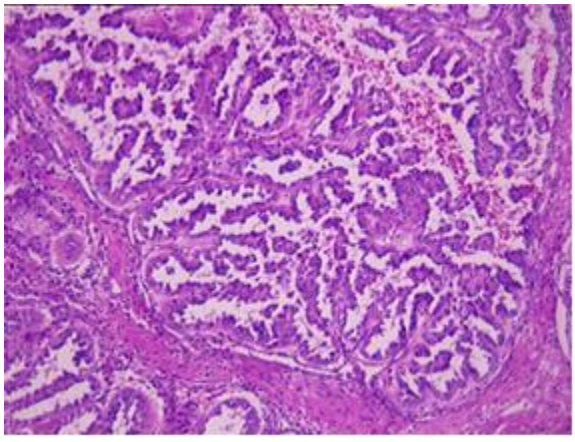


FIGURE 23 : Tumour cells in papillary pattern with infiltration (100X) HPE -638/10

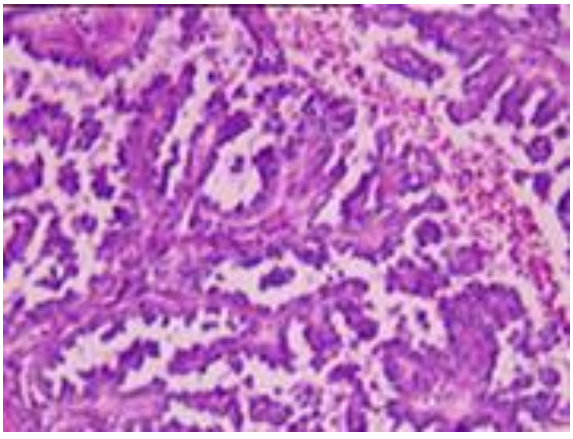


FIGURE 24 : Cells in delicate papillary pattern with fibro – vascular core (400X) HPE – 638/10

MUCINOUS ADENOCARCINOMA

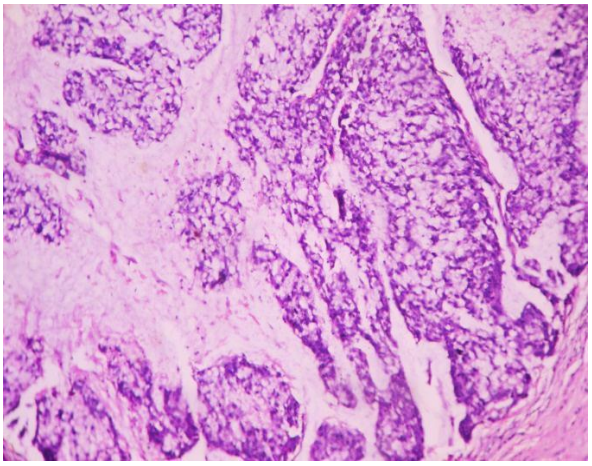


FIGURE 25 : Chains and sheets of malignant cells floating in extra – cellular mucin pool (100X) HPE – 5315/10

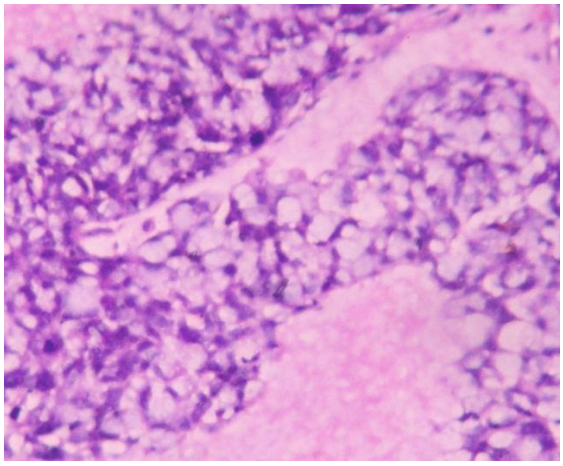


FIGURE 26 : Malignant epithelial cells with pleomorphism and scattered signet ring cells (400X) HPE – 5315/10

SIGNET RING CELL ADENOCARCINOMA

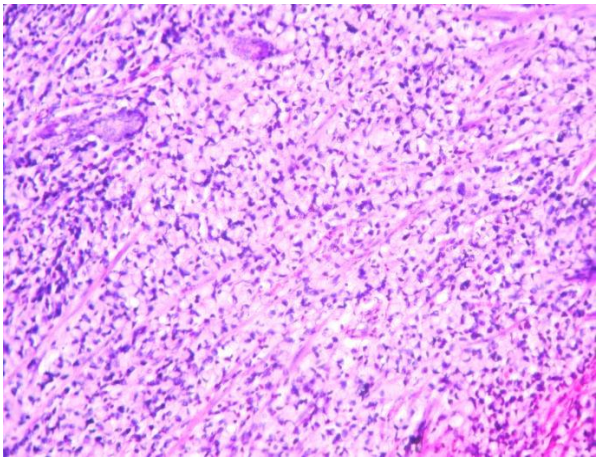


FIGURE 27 : Sheets of signet ring cells forming >50% of the tumour. (100X) HPE – 4691/10

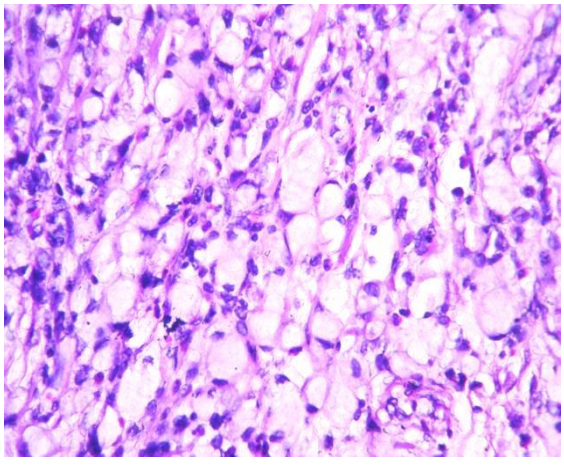


FIGURE 28 : Sheets of malignant cells with abundant intra-cytoplasmic mucin. (400X) HPE – 4691/10

SQUAMOUS CELL CARCINOMA

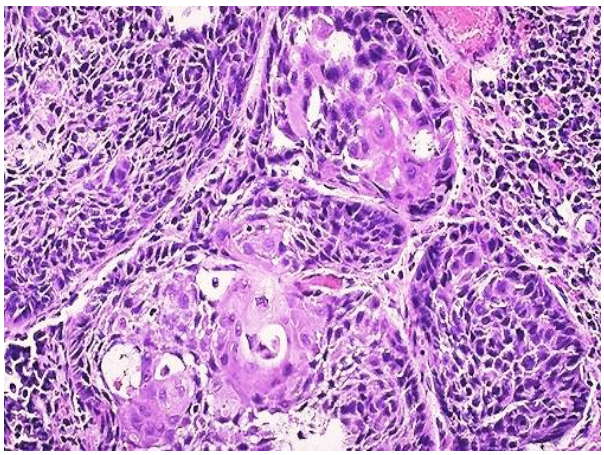


FIGURE 29 : Moderately differentiated SCC (100X) HPE – 8586/10

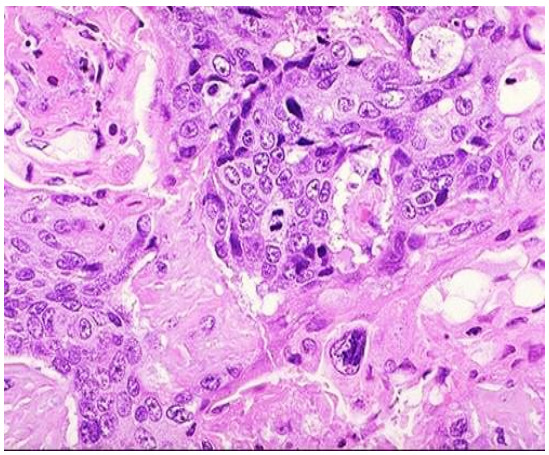


FIGURE 30 : Tumour cells resembling squamous cells with intra-cellular keratin (400X) HPE – 8586/10

INFILTRATION INTO MUSCULARIS PROPRIA

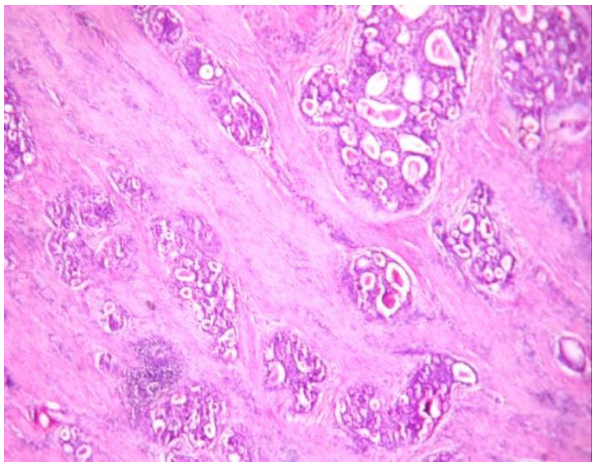


FIGURE 31: IT adenocarcinoma infiltrating the muscularis proper (100X) HPE – 921/10

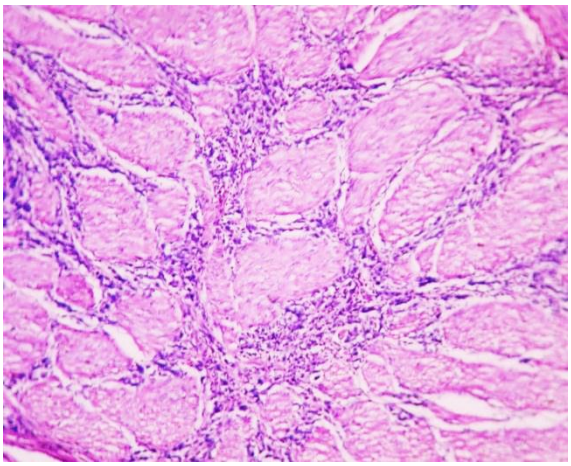


FIGURE 32: DT adenocarcinoma infiltrating the muscularis proper (100X) HPE – 2456/10

OTHER PROGNOSTIC FACTORS

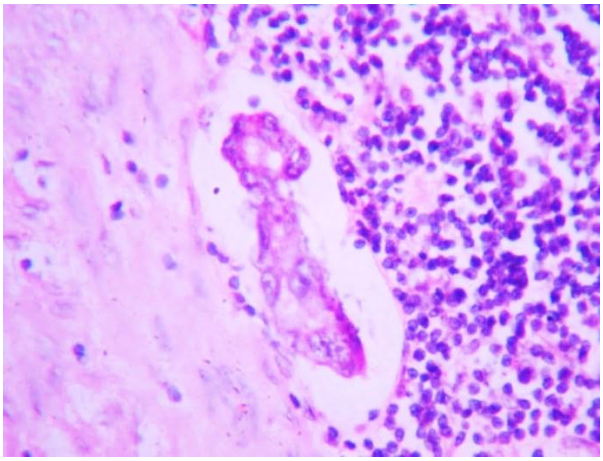


FIGURE 33 : Lymphatic invasion (400X)
HPE – 151/10

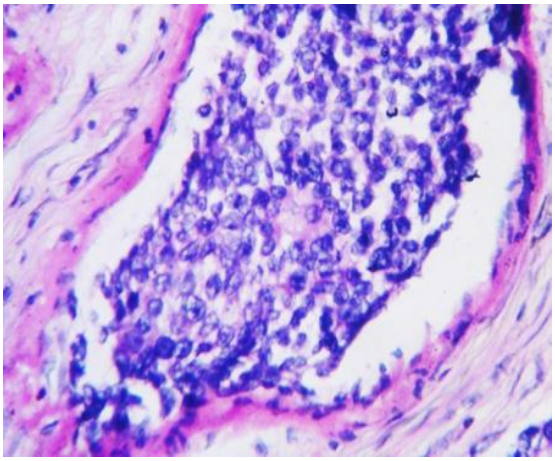


FIGURE 34 : Vascular invasion (400X)
HPE - 2130/10

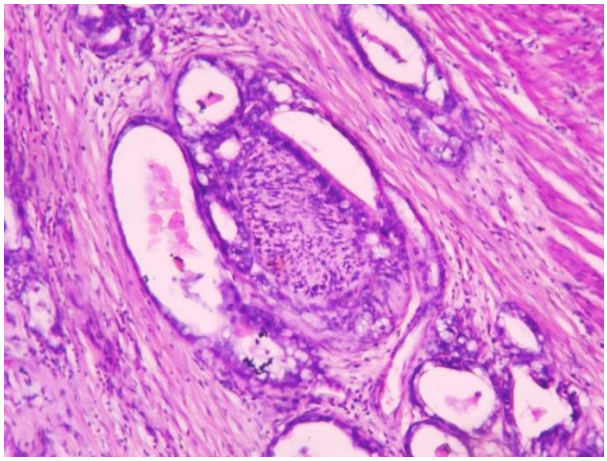


FIGURE 35 : Perineural infiltration (100X)
HPE – 6093/10

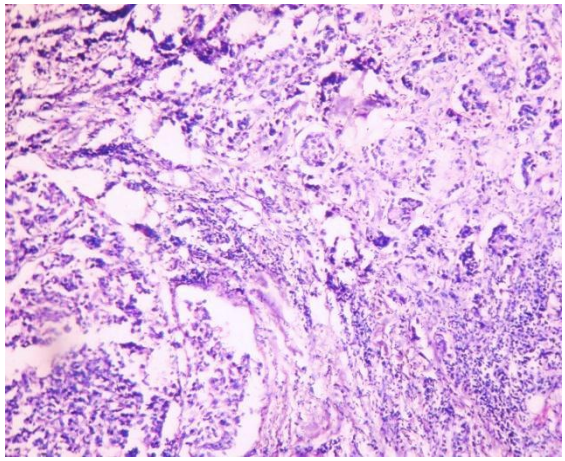


FIGURE 36 : Lymphocytic infiltration (100X) HPE – 4821/10

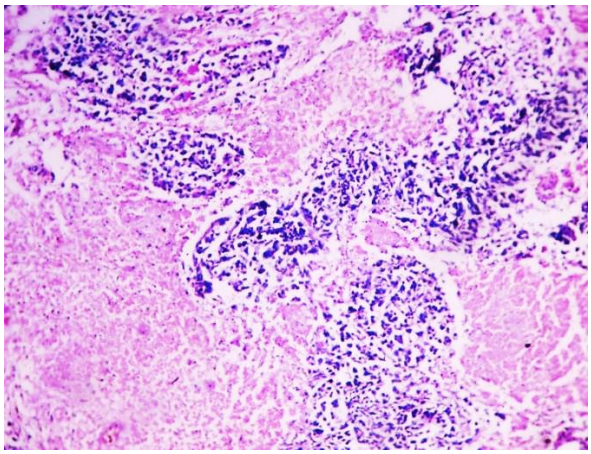


FIGURE 37 : Necrosis (100X) HPE – 4821/10

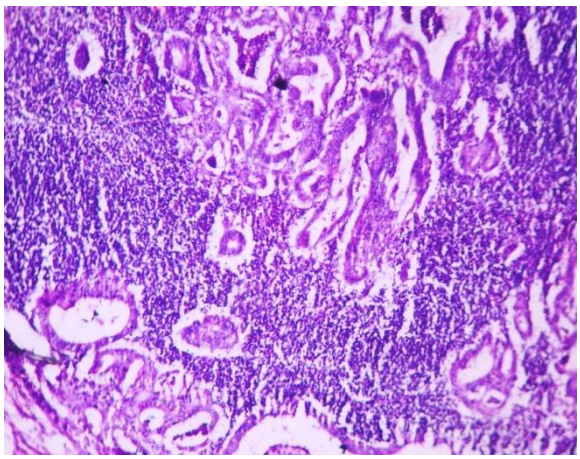


FIGURE 38 : Metastatic deposit in node (100X) HPE – 4983/10

KRUKENBERG TUMOUR

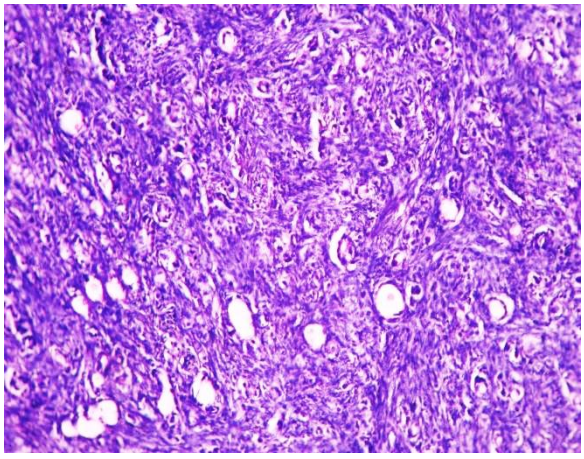


FIGURE 39 : Malignant glands within the ovarian stroma (100X) HPE – 3729/10

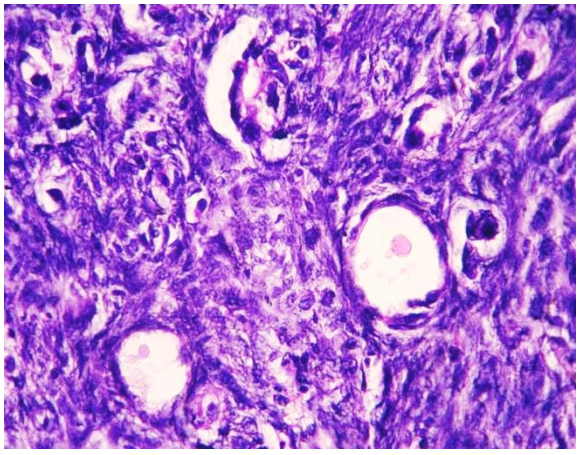


FIGURE 40 : Malignant glands lined by cuboidal cells showing pleomorphism (400X) HPE – 3729/10

p53 POSITIVE

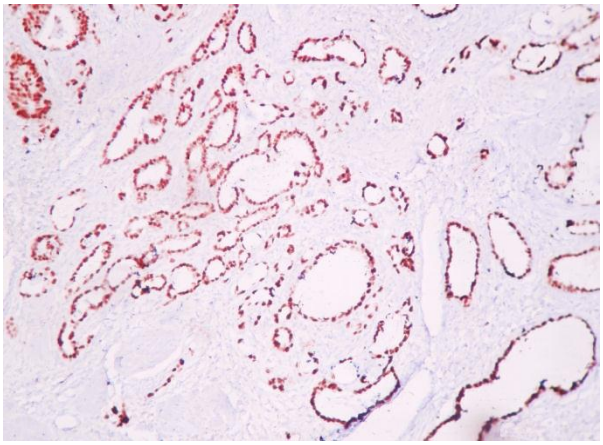


FIGURE 41 : Gastric adenocarcinoma – IT Grade I – Strong nuclear positivity for p53 in >90% of tumour nuclei. (100X) HPE – 7127/10

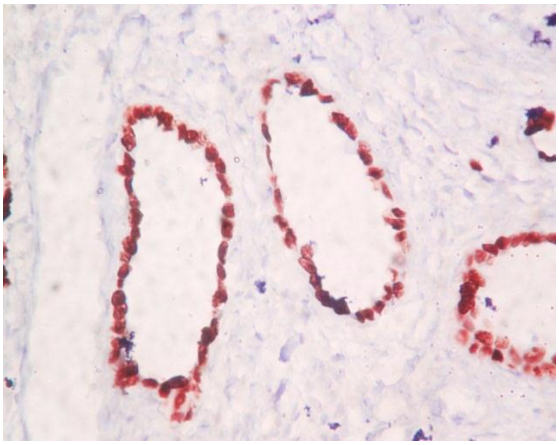


FIGURE 42 : Strong nuclear staining for p53 in > 90% of tumour nuclei. (400X) HPE – 7127/10

p53 NEGATIVE

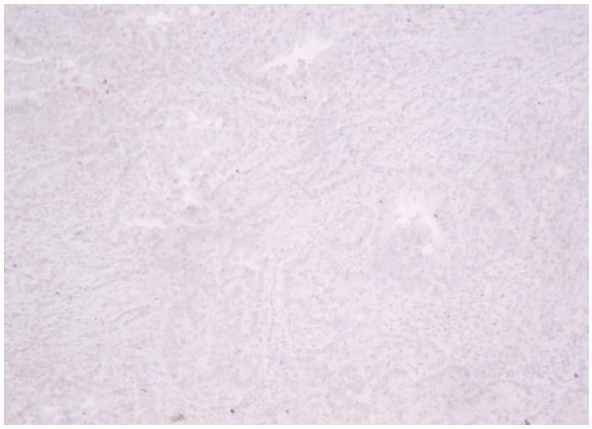


FIGURE 43 : Gastric adenocarcinoma – IT Grade I – Very weak nuclear staining for p53 In < 5% tumour nuclei. (100X) HPE – 7165/10

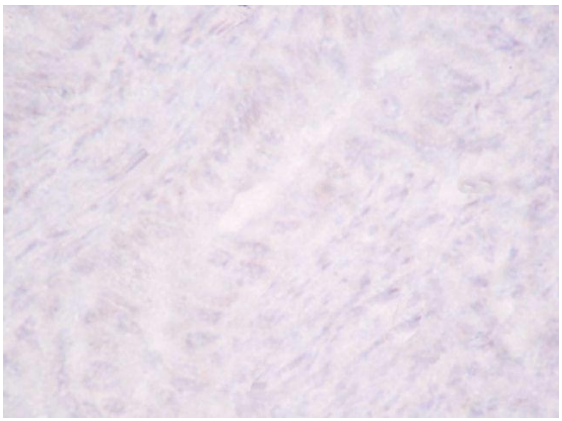


FIGURE 44 : Very weak nuclear staining for p53 in < 5% tumour nuclei. (400X) HPE – 7165/10

p53 POSITIVE

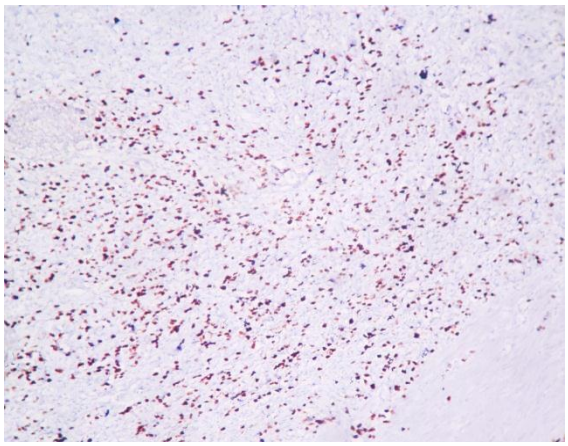


FIGURE 45 : Gastric adenocarcinoma – DT - Strong nuclear staining for p53 in 80% of Tumour nuclei (100X) HPE – 2922/10

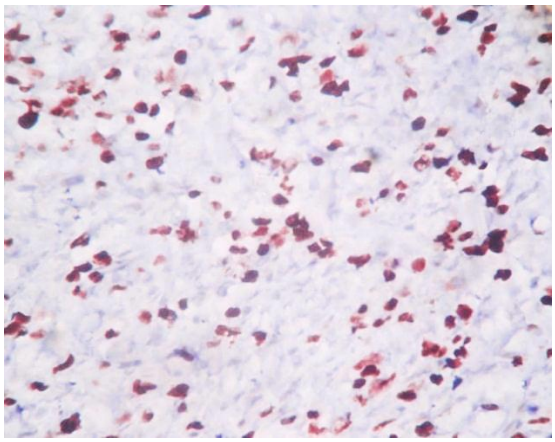


FIGURE 46 : Strong nuclear staining for p53 in 80% of tumour nuclei (400X) HPE – 2922/10

p53 NEGATIVE

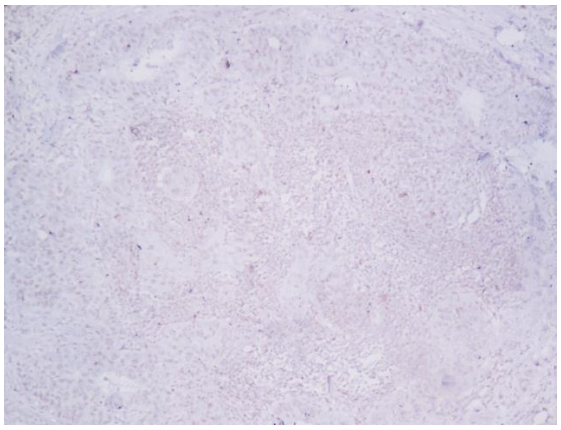


FIGURE 47 : Gastric adenocarcinoma – DT Very weak nuclear staining for p53 in <10% tumour nuclei. (100X) HPE – 2906/10

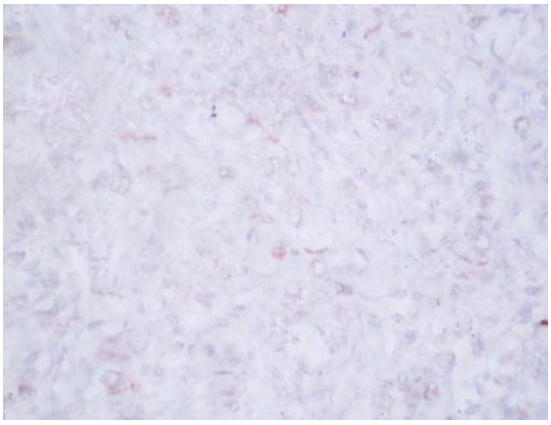


FIGURE 48 : Very weak nuclear staining for p53 in <10% of tumour nuclei (400X) HPE – 2906/10

HIGH Ki-67 LABELING INDEX

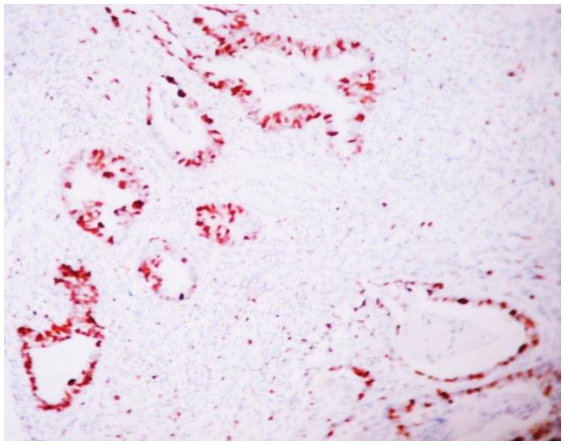


FIGURE 49 : Gastric adenocarcinoma – IT Grade II – Strong nuclear staining for Ki-67 in 645/1000 tumour nuclei. Ki-67 LI – 64.5%. (100X) HPE – 8755/10

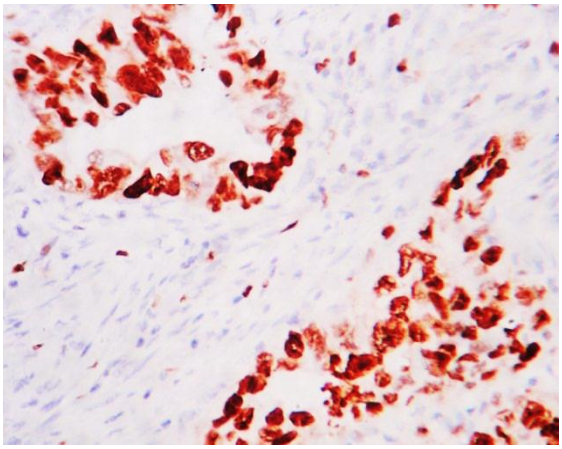


FIGURE 50 : Strong nuclear staining for Ki-67 in 654/1000 tumour nuclei . Ki-67 LI – 64.5% (400X) HPE – 8755/10

LOW Ki-67 LABELING INDEX

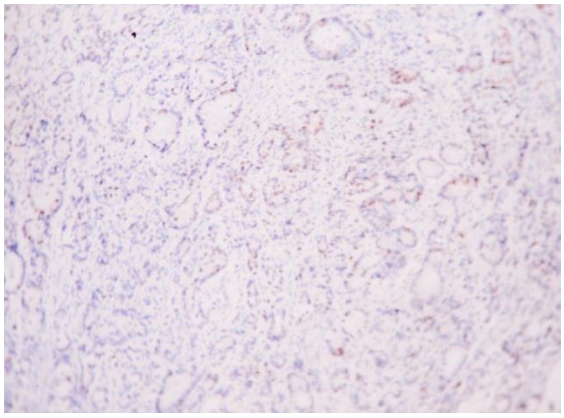


FIGURE 51 : Gastric adenocarcinoma – IT Grade II – moderate nuclear staining for Ki-67 in 105/1000 tumour nuclei. KI-67 LI – 10.5% (100X) HPE – 3667/10

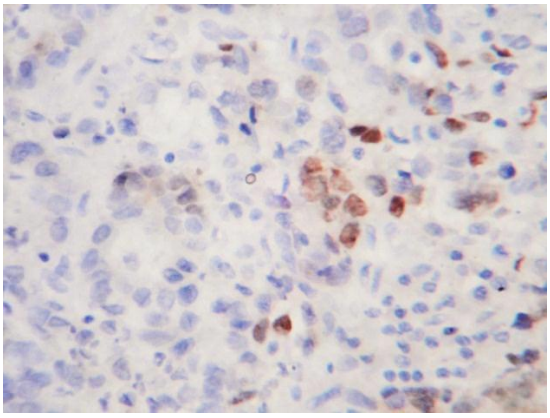


FIGURE 52 : Moderate nuclear staining for Ki-67 in 105/1000 tumour nuclei. Ki-67 LI – 10.5% (400X) HPE – 3667/10

HIGH Ki-67 LABELING INDEX

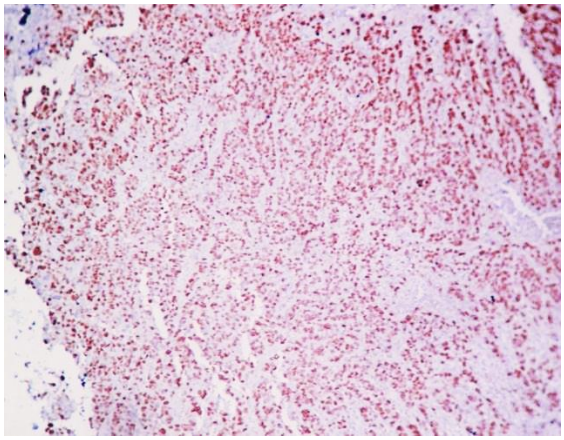


FIGURE 53 : Gastric adenocarcinoma – DT - Strong nuclear positivity for Ki-67 in 502/1000 tumour nuclei. Ki-67 LI – 50.2% (100X) HPE – 4501/10

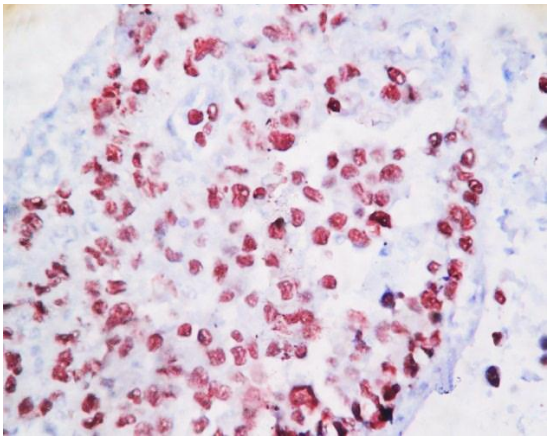


FIGURE 54 : Strong nuclear positivity for Ki-67 in 502/1000 tumour nuclei. Ki-67 LI – 50.2%. (400X) HPE- 4501/10

LOW Ki-67 LABELING INDEX

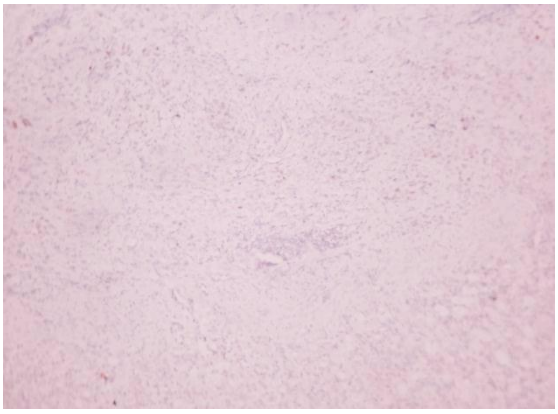


FIGURE 55 : Gastric adenocarcinoma – DT - Weak nuclear stain for Ki-67 in 69/1000 Tumour nuclei. Ki-67 LI – 6.9% (100X) HPE – 2906/10

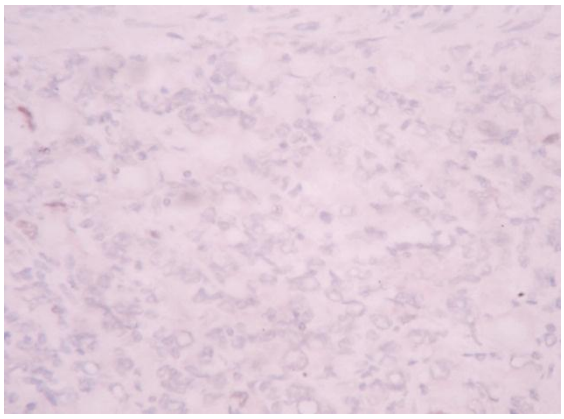


FIGURE 56 : Weak nuclear stain for Ki-67 in 69/1000 tumour nuclei. Ki-67 LI – 6.9% (400X) HPE – 2906/10

p53 POSITIVE

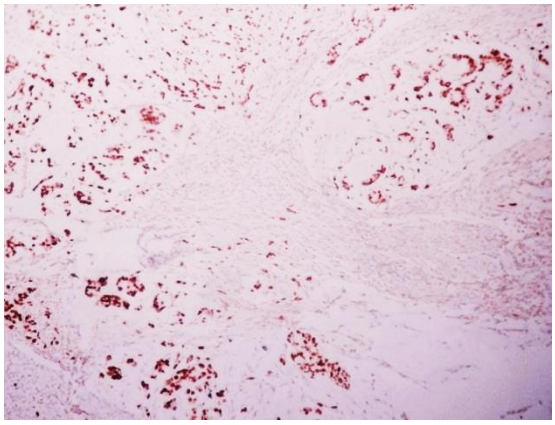


FIGURE 57 : Mucinous carcinoma – strong nuclear positivity for p53 in >80% tumour nuclei. (100X) HPE – 399/10

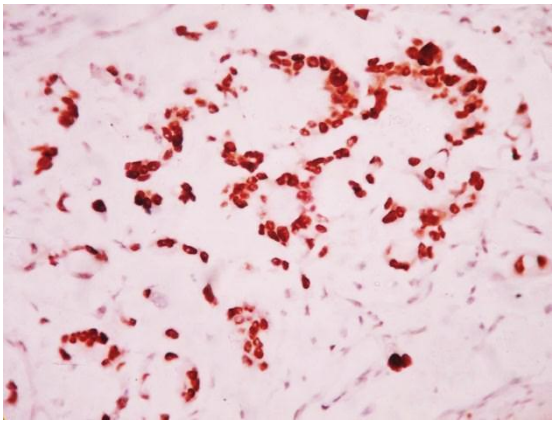


FIGURE 58 : Strong nuclear positivity for p53 in >80% tumour nuclei. (400X) HPE – 399/10

HIGH Ki-67 LABELING INDEX

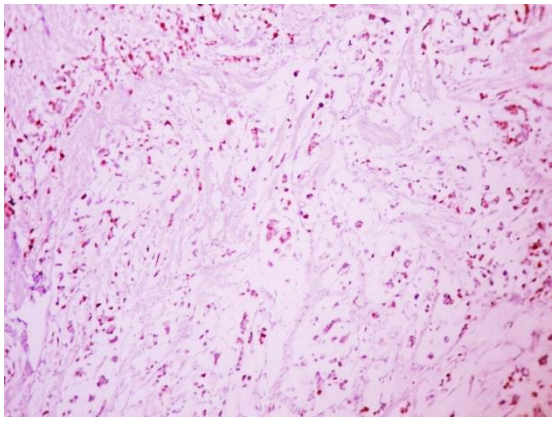


FIGURE 59: Mucinous carcinoma – Strong nuclear positivity for Ki-67 in 566/1000 tumour nuclei. Ki-67 LI – 56.6% (100X) HPE – 399/10

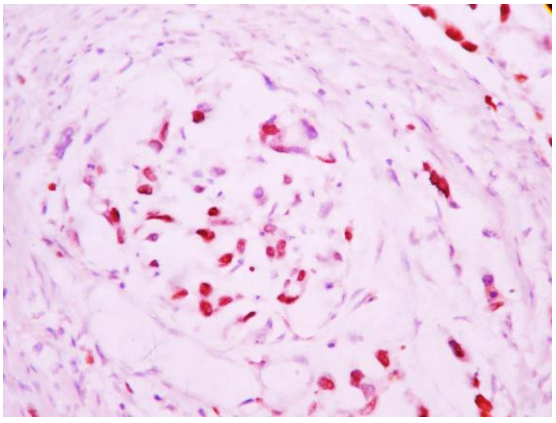


FIGURE 60 : Strong nuclear positivity for Ki-67 in 566/1000 tumour nuclei. Ki67 LI - 56.6% (400X) HPE – 399/10

CHART 1 AGE AND SEX WISE DISTRIBUTION OF GASTRIC CARCINOMA

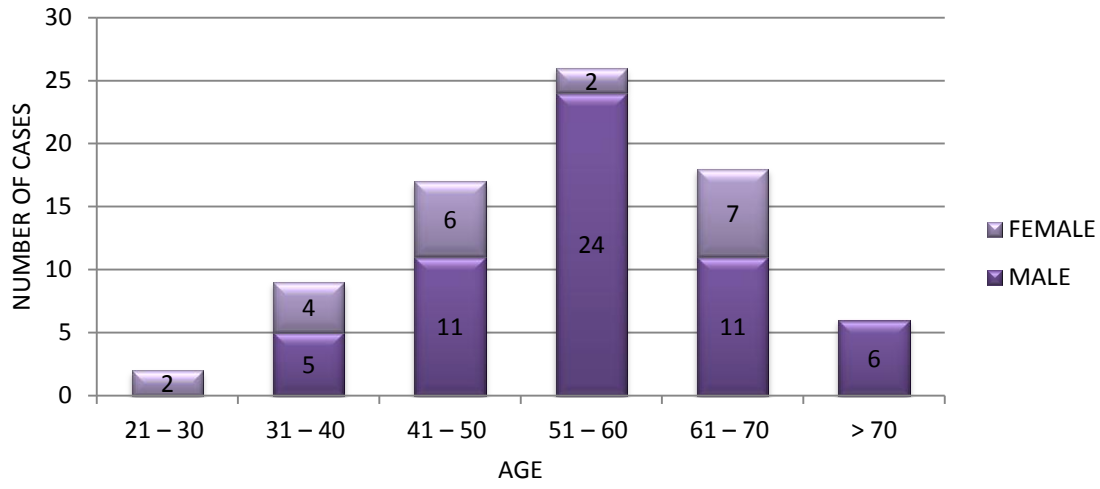


CHART 2 - SITE OF GASTRIC CANCER

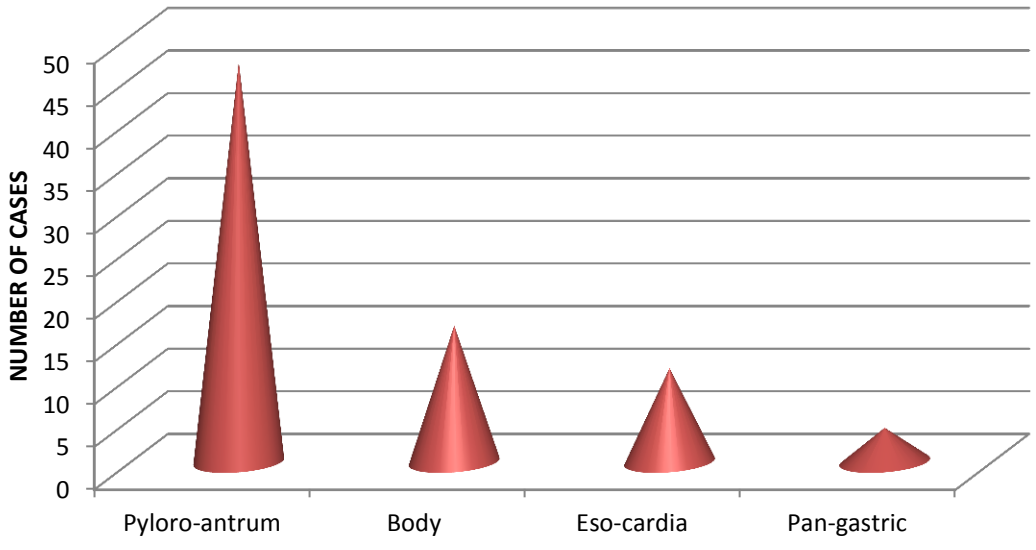


CHART 3 -DISTRIBUTION OF GASTRIC CARCINOMA ACCORDING TO GROSS MORPHOLOGY

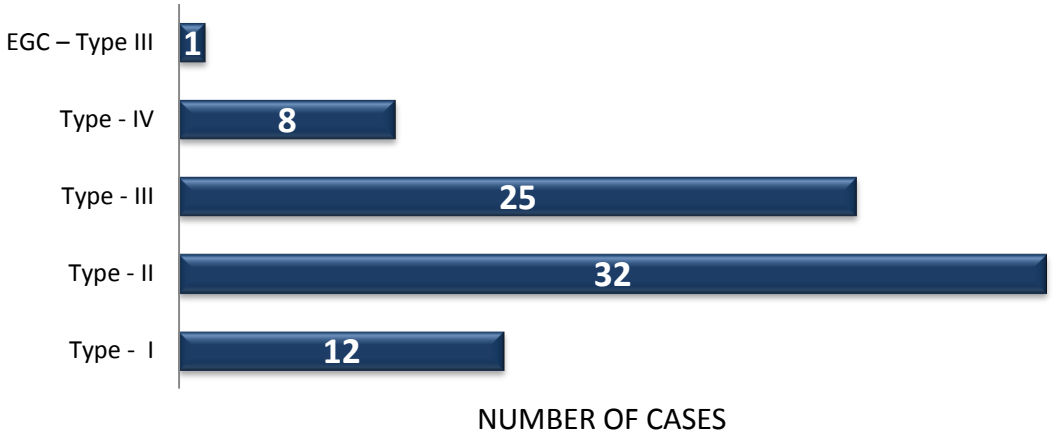


CHART 4 - DISTRIBUTION OF SIZE IN GASTRIC CARCINOMA

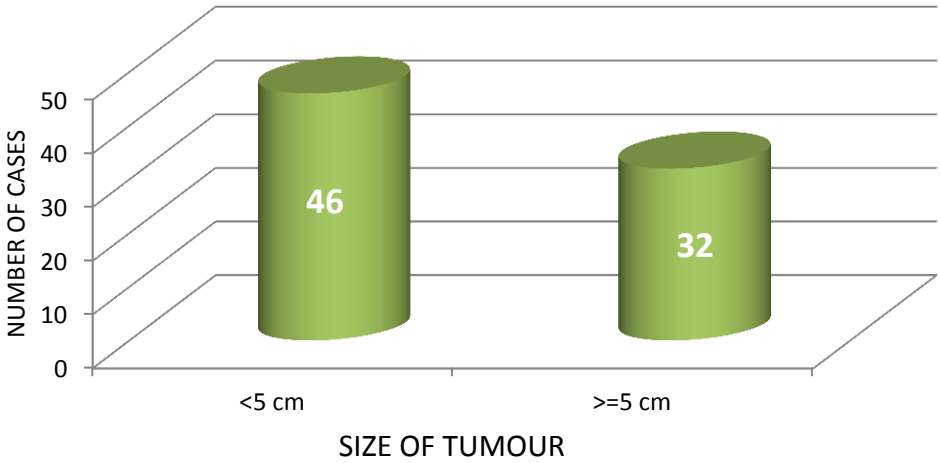


CHART 5 DISTRIBUTIONS OF HISTOLOGICAL SUBTYPES OF GASTRIC CANCERS

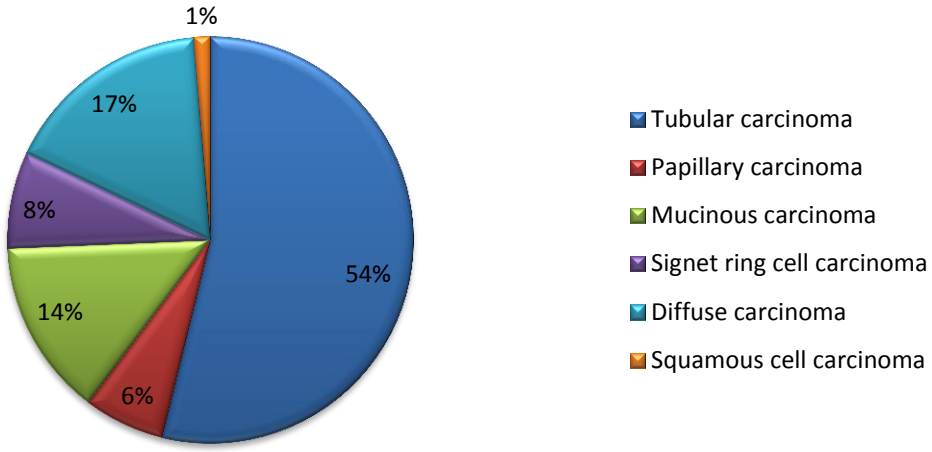


CHART 6 - DISTRIBUTION OF GASTRIC CANCER ACCORDING TO LAUREN'S CLASSIFICATION

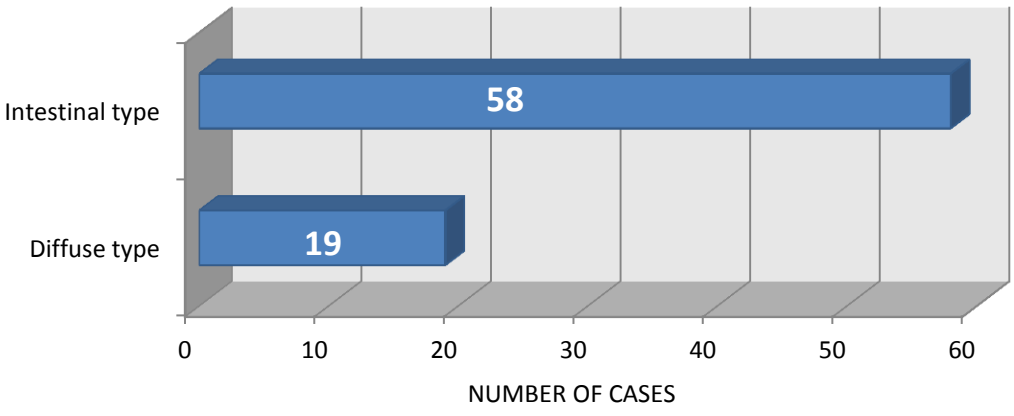


CHART 7 - DISTRIBUTION OF HISTOLOGICAL GRADE IN GASTRIC CARCINOMAS

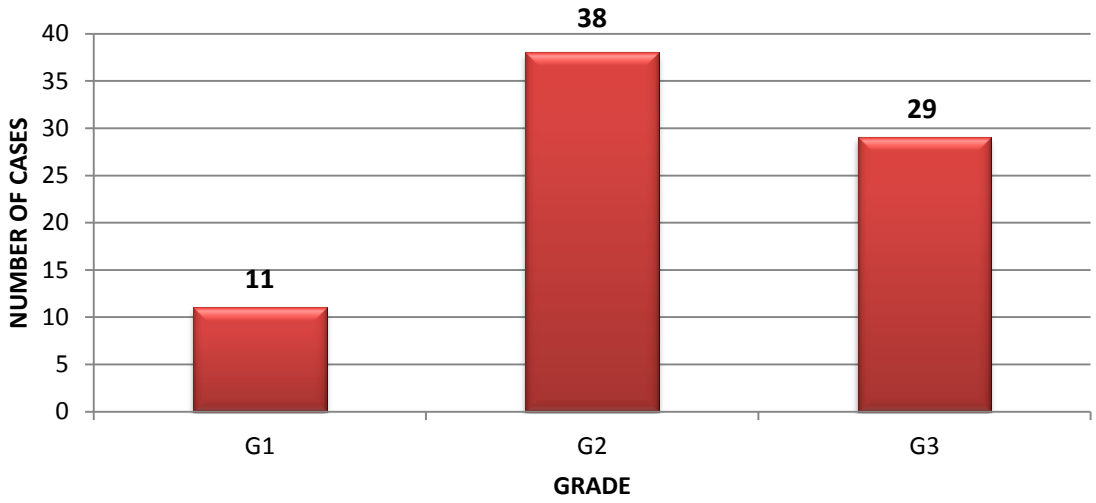


CHART 8 - DISTRIBUTION OF GASTRIC CARCINOMAS ACCORDING TO DEPTH OF INVASION

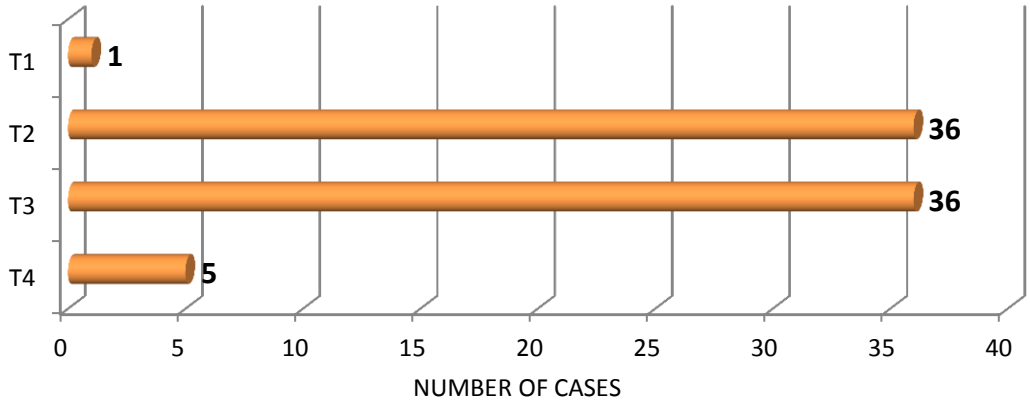


CHART 9 - DISTRIBUTION OF LYMPH NODE METASTASIS IN GASTRIC CANCERS

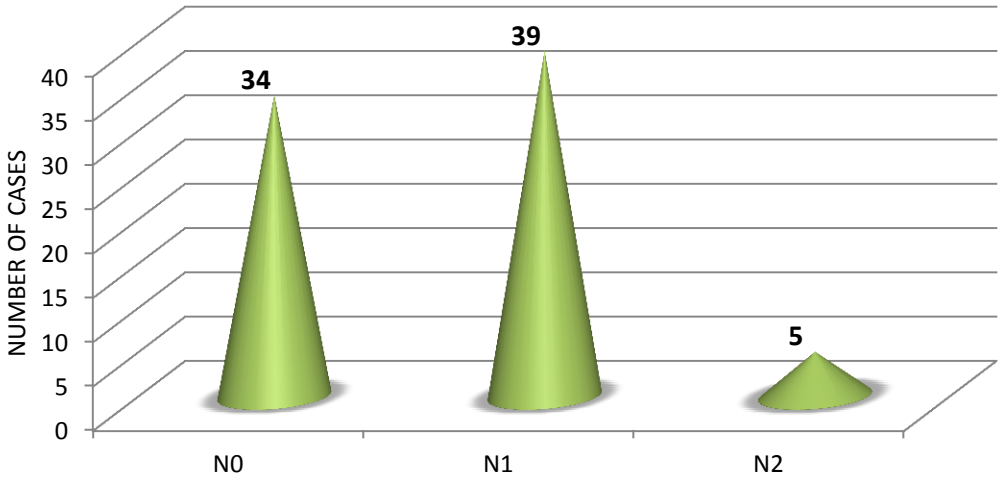


CHART 10 - DISTRIBUTION OF GASTRIC CARCINOMA ACCORDING TO STAGE

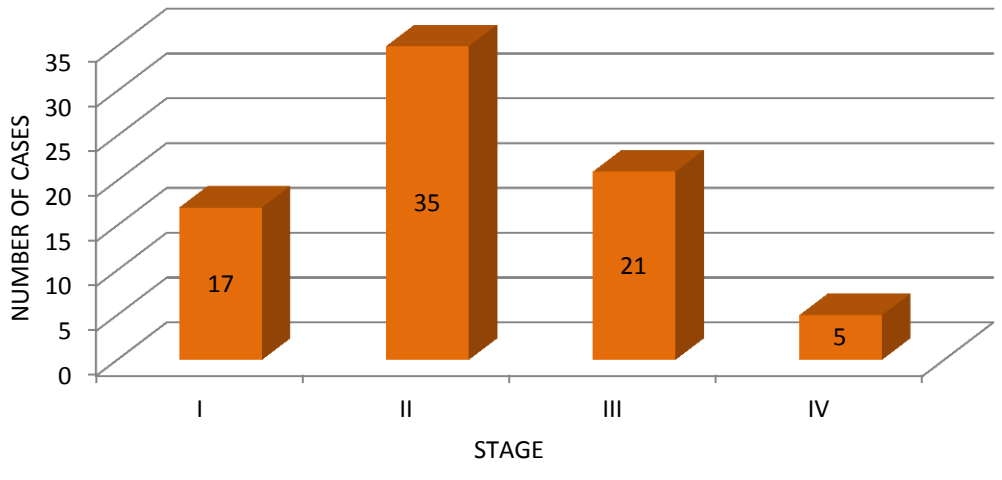


CHART 11 - DISTRIBUTION OF OTHER PROGNOSTIC FACTORS IN GASTRIC CARCINOMA

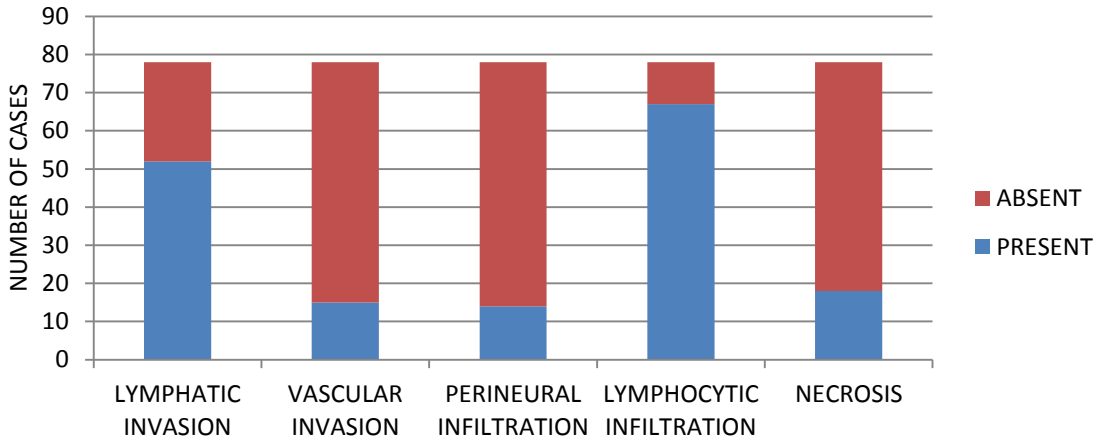


CHART 12 - DISTRIBUTION OF P53 EXPRESSION AND Ki - 67 LABELING INDEX IN GASTRIC CARCINOMAChart Title

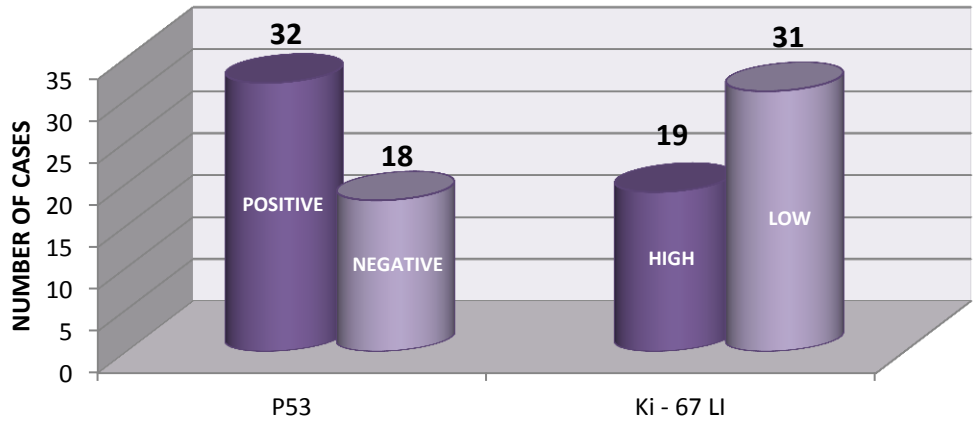


CHART 13 - AGE VS P53 EXPRESSION AND KI-67 LI

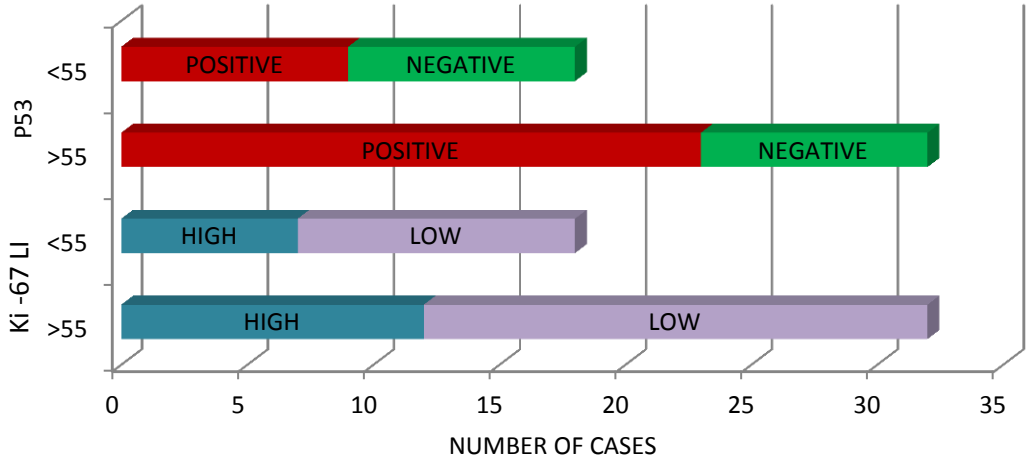


CHART 14 - GENDER VS P53 EXPRESSION AND Ki-67 LI

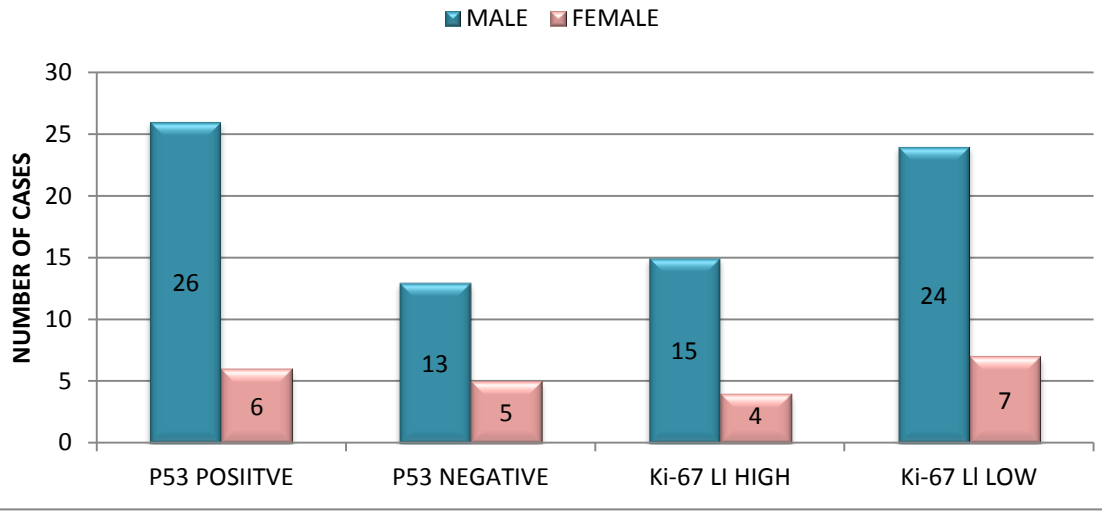


CHART 15 - SITE VS P53 EXPRESSION AND Ki-67 LI

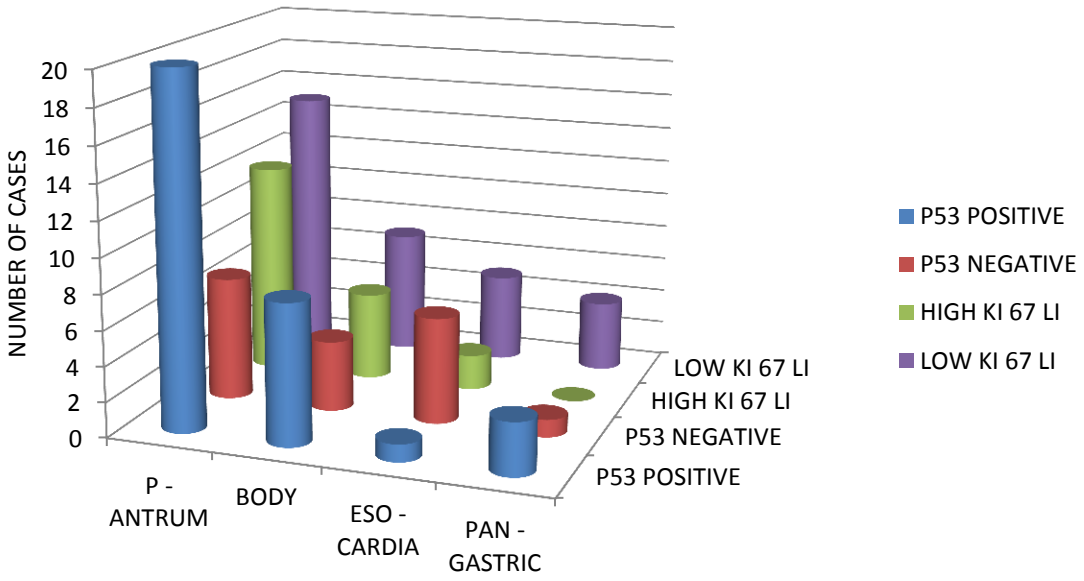


CHART 16 - GROSS TYPE VS P53 EXPRESSION AND Ki-67 LI

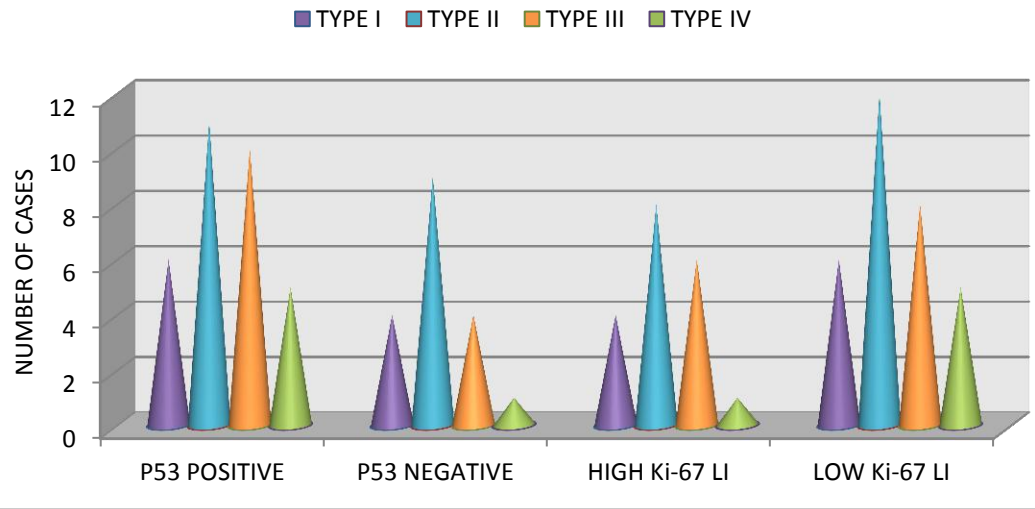


CHART 17 - SIZE VS P53 EXPRESSION AND Ki-67 LI Title

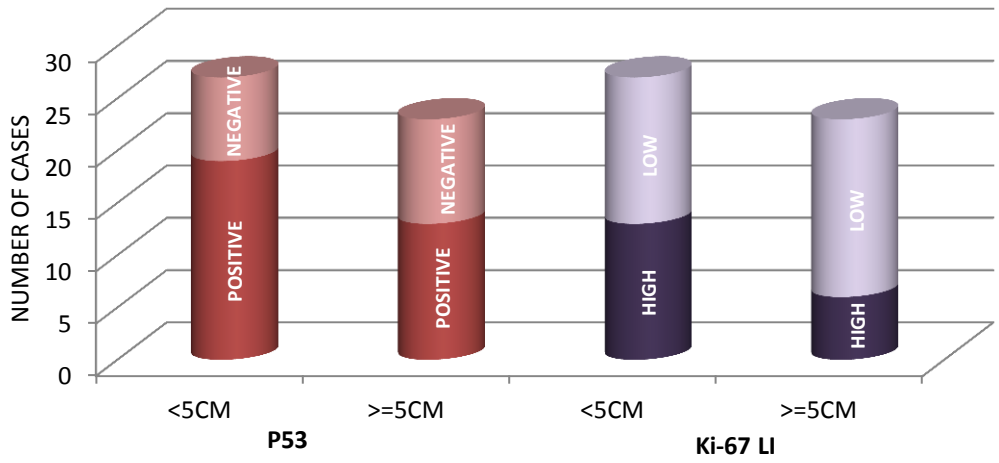


CHART 18 - HISTOLOGICAL TYPE VS P53 EXPRESSION AND Ki-67 LI

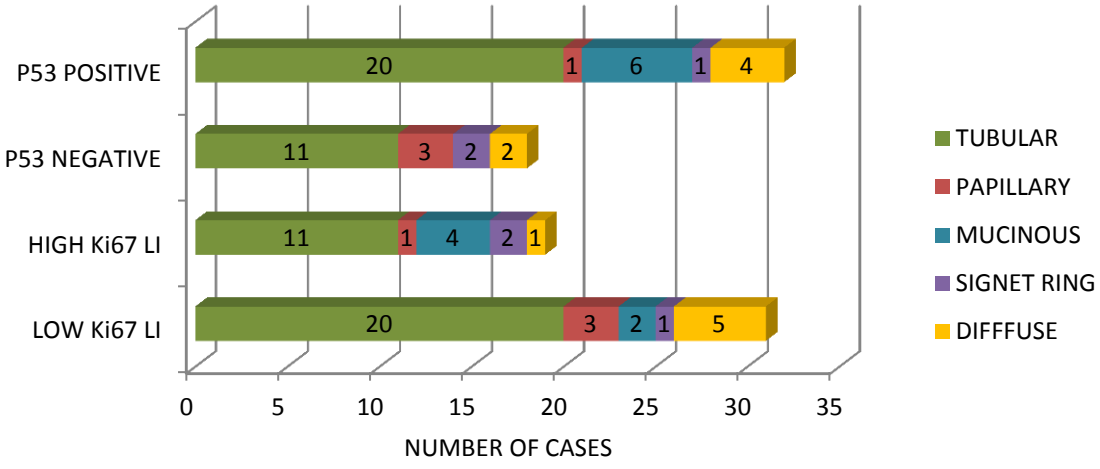


CHART 19 - LAUREN'S TYPE VS P53 EXPRESSION AND Ki-67 LI

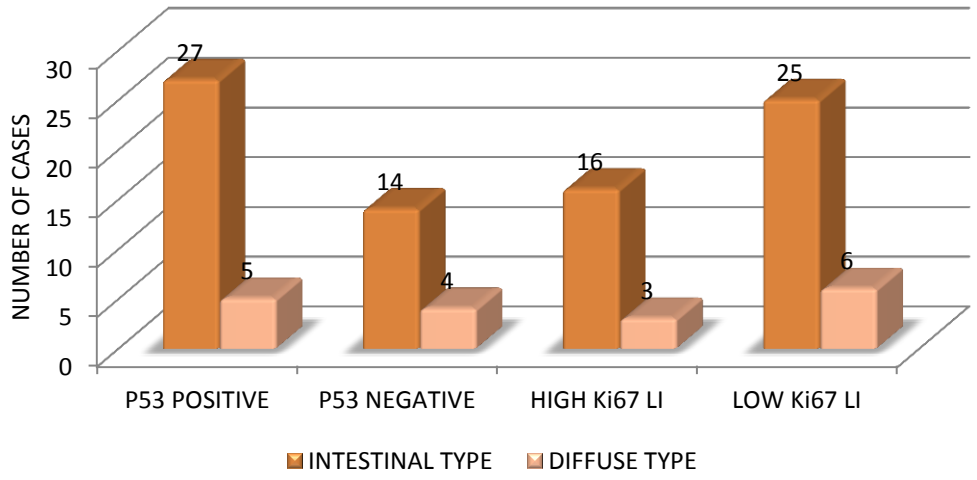


CHART 20 - GRADE VS P53 EXPRESSION AND Ki-67 LI

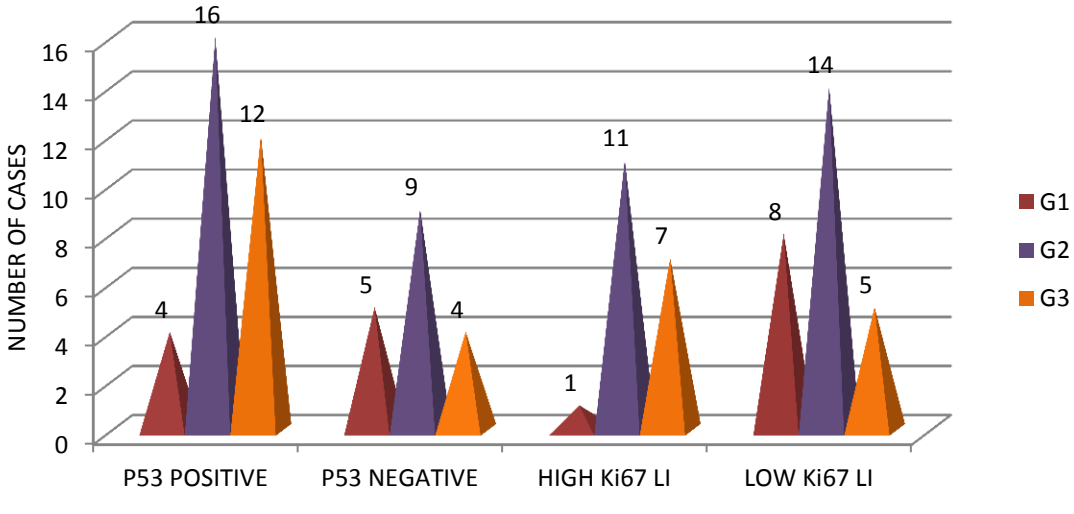


CHART 21 - DEPTH OF INVASION VS P53 EXPRESSION AND Ki-67 LI

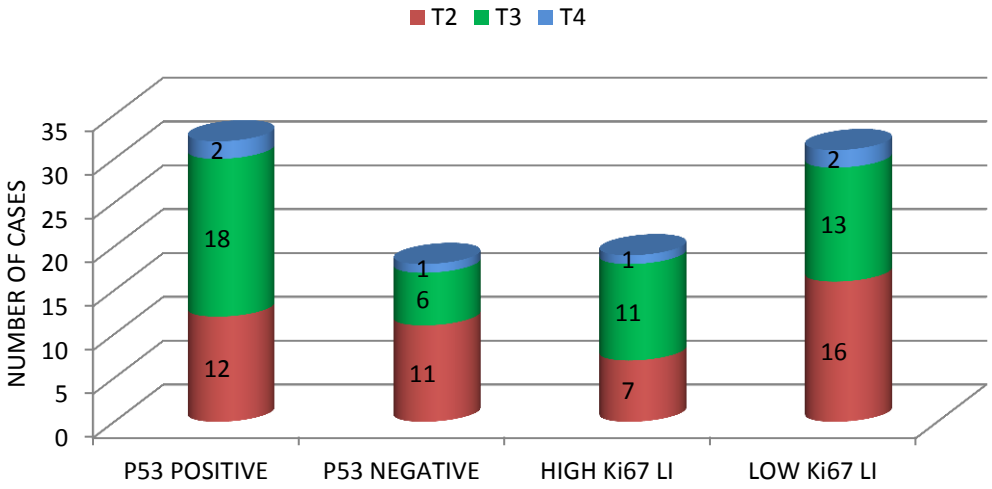


CHART 22 - N STAGE VS P53 EXPRESSION AND Ki-67 LI

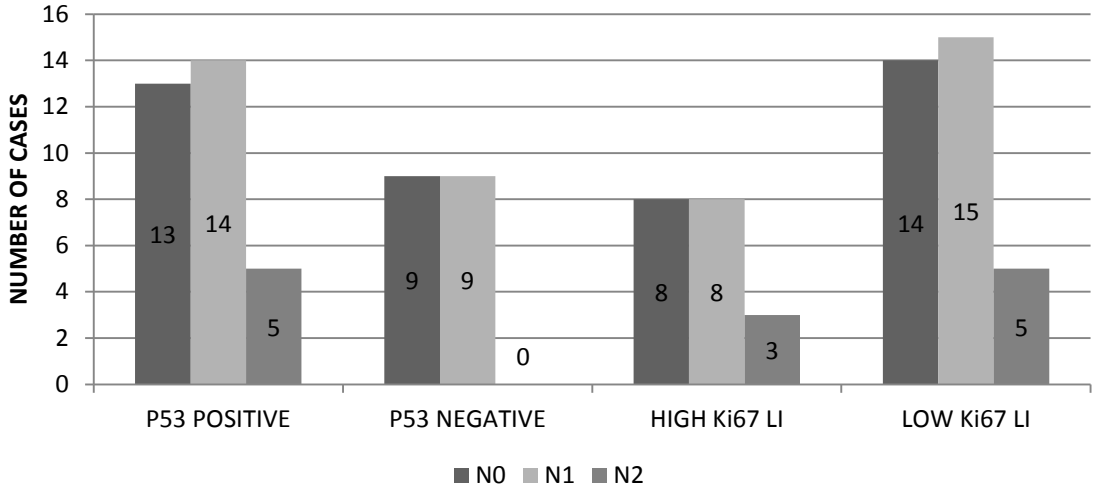


CHART 23 - TNM STAGE VS P53 EXPRESSION AND Ki-67 LI

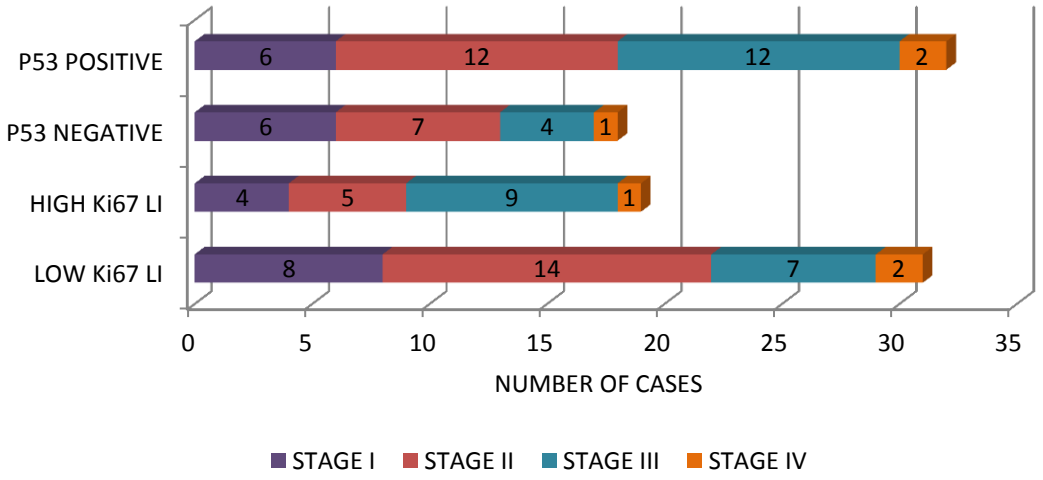


CHART 24 - P53 EXPRESSION VS Ki-67 LI

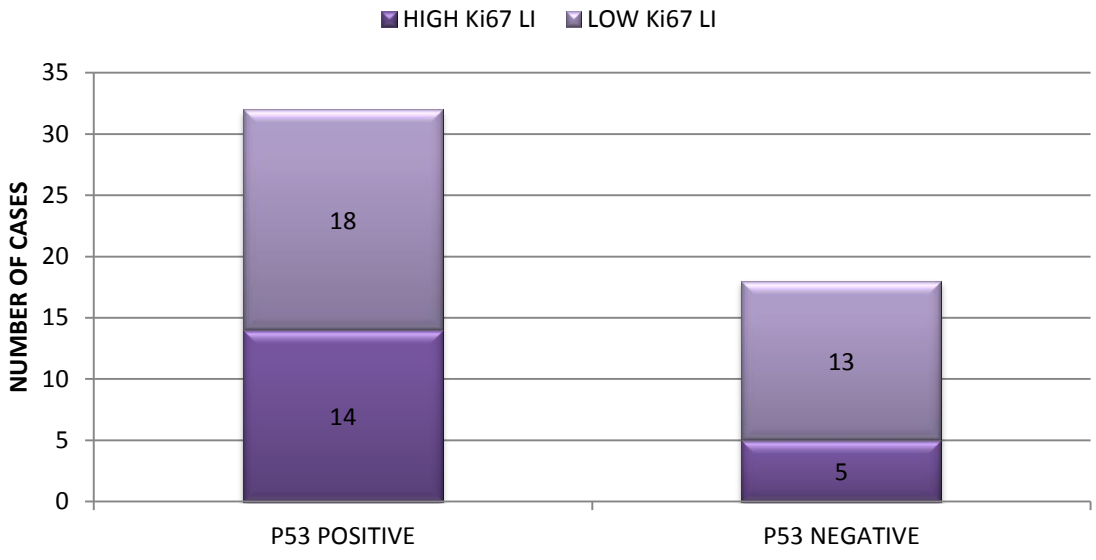
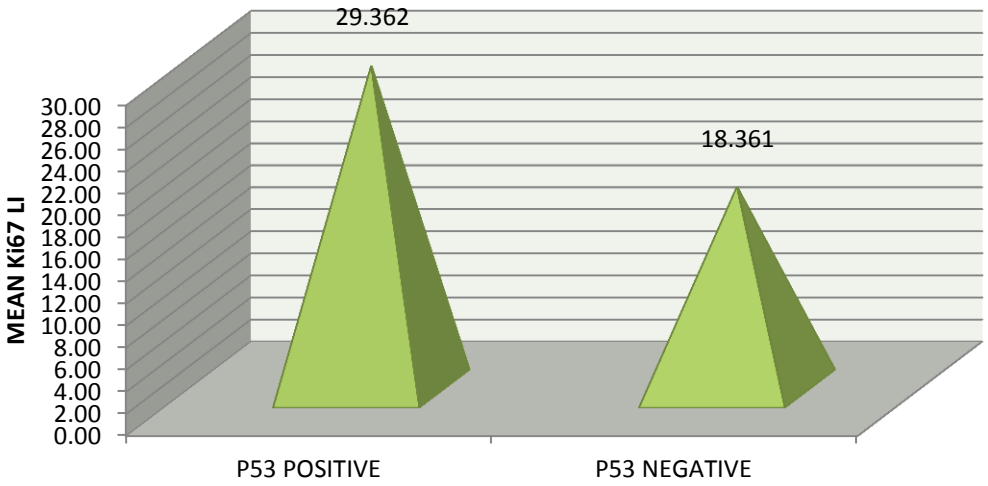


CHART 25 - MEAN Ki67 LI IN P53 POSITIVE AND NEGATIVE TUMOURS



DISCUSSION

DISCUSSION

Gastric cancer is a life threatening disease and represents a significant health problem worldwide. It is the second most common cancer worldwide and the third most common cancer in India[1]. The incidence of gastric cancer in Chennai is about 13.6 per lakh in men and 6.5 per lakh in women[28].

Many biological markers have been examined as possible tools for the evaluation of the biological behavior of gastric cancer in order to predict the clinical outcome. Among these, immunohistochemical staining of cell cycle regulator p53 and proliferation marker Ki-67 have been proposed to be of prognostic value.

In the present study, immunohistochemical evaluation was done in 50 cases of gastric carcinomas and an attempt was made to correlate the p53 expression and Ki-67 labeling index with the known prognostic factors of gastric cancers.

Madras Medical College being a tertiary referral centre, about 3.76% of gastric cancers was reported among the specimens received in the year 2010. Among the entire gastric specimens received for histopathological examination, 54.39% of the cases were reported to be malignant.

This study showed the age of gastric cancer patients ranged from 28 years to 75 years with the mean age of 55.04 years. The highest incidence of gastric cancer occurred in 51 to 60 year age group. This is in concurrence with the

study done by Y.E.Joo et al[100] who observed a mean age of 58.7 years with a range from 28 to 79 years. In the study done by Nobuyuki Igarashi et al[101] the incidence of gastric cancer in men and women was 74.1% and 25.9% respectively. In concurrence with the above study, a significant predominance of gastric cancers in men who accounted for 78% of the cases and women accounted for 22% was observed.

The most common site of gastric cancer in this study is the pyloro – antrum (54%). This is almost similar to the study of N.E. Tzanakis et al[102] and Daniela Lazar et al[103]. In their study, Tzanakis et al[102] observed 51.6% tumours in the antrum and Daniela Lazar et al[103] observed 50.8% tumours in the antrum (Table 40).

TABLE 40 – COMPARISON OF DISTRIBUTION OF GASTRIC TUMOUR LOCATION

Tumour location	N.E.Tzanakis et al[102]	Daniela Lazar et al[103]	Czyzewska J et al[108]	Current study
Antrum	51.6%	50.8%	60%	54%
Body	34.4%	24.5%	20%	24%
Eso – cardia	14%	13.1%	15.6%	14%
Pan - gastric	-	11.4%	4.4%	8%

Daniela Lazar et al[103] observed 8.2% of Borrmann type I tumours, 32.7% of type II tumours, 36% of type III tumours and 14.7% of type IV tumours. Similar results were observed with 20% of type I tumours, 40% of type II

tumours, 28% of type III tumours and 12% of type IV tumours. Similar findings were also observed by Jurgen et al[104].

In this study, an average tumour size of 5 cm was observed which was similar to the findings observed by Y.E.Joo[100] et al and Tzanakis et al[102]. Y.E Joo observed an average tumour size of 5.2 cm and Tzanakis et al[102] observed an average tumour size of 5.1 cm.

The most common histological subtype of gastric cancer in this study is Tubular carcinoma. This is almost similar to the study of Daniela Lazar et al[103] and Y.Kakeji et al[105] (Table 41).

TABLE 41 – COMPARISON OF DISTRIBUTION OF HISTOLOGICAL TYPES OF GASTRIC CARCINOMA

Histological type	Daniela et al[103]	Kakeji et al[105]	Current study
Tubular carcinoma	45.9%	89.5%	62%
Papillary carcinoma	8.2%	2%	8%
Mucinous carcinoma	13.1%	5.2%	12%
Signet ring cell carcinoma	27.8%	3.1%	6%
Diffuse carcinoma	4.9%	-	12%

The most common histological subtype (Lauren's) in this study was the Intestinal type (82%). This is similar to observations made by Casasola et al[106] wherein, intestinal type accounted for 81.9% and diffuse type accounted for 18.1% (Table 42).

TABLE 42 – COMPARISON OF LAUREN"S HISTOLOGICAL TYPE

Lauren's type	Czyzewska et al[108]	Daniela et al[103]	Casasola et al[106]	Current study
Intestinal type	75.5%	72.1%	81.9%	82%
Diffuse type	24.5%	27.9%	18.1%	18%

In the present study, the G2 (moderately differentiated) tumours were more common than the other grades of distribution. This was in concurrence with the study conducted by Casasola et al[106] (Table 43).

TABLE 43 – COMPARISON OF GRADE OF TUMOUR

Grade	Casasola et al[106]	Tzanakis et al[102]	Daniela et al[103]	Current study
G1	16%	5.4%	3.2%	18%
G2	74.6%	22.6%	32.8%	50%
G3	9.4%	69.9%	64%	32%

A higher proportion of T3 tumours, closely followed by T2 tumours were observed in this study, similar to the studies of Giovanni de Manzoni et al[107], and Y.E. Joo[100] et al (Table 44).

TABLE 44 – COMPARISON OF DEPTH OF TUMOUR

Depth	T1	T2	T3	T4
Giovanni et al[107]	-	25%	66%	9%
Y.E.Joo et al[100]	13.4%	24.3%	51.2%	11.1%
Daniela et al[103]	6.5%	14.7%	27.8%	49.2%
Jurgen et al[104]	16.9%	36.6%	38.6%	7.9%
Current study	-	46%	48%	6%

There was nodal metastasis in 56% of the cases and no nodal metastasis in 44% of cases. This was similar to the study by Y.E Joo[100] et al who observed nodal metastasis in 51.3% cases and no nodal metastasis in 48.7% cases & the study by Czyzewska J et al[108] who observed nodal metastasis in 55.6% and no nodal metastasis in 44.4% (Table 45).

TABLE 45 – COMPARISON OF NODAL METASTASIS

Nodal status	N0	N1	N2	N3
Giovanni et al[107]	21.4%	35.7%	42.9%	-
Daniela et al[103]	29.5%	26.2%	37.8%	6.5%
Jurgen et al[104]	32.4%	22%	45.6%	-
Current study	44%	46%	10%	-

Most of the cases presented in stage II followed by closely followed by stage III in this study. This did not concur with the other studies which showed a predominance of stage IV tumours (Table 46)

TABLE 46 COMPARISON OF STAGE OF GASTRIC TUMOUR

Stage	Daniela et al[103]	Y.E. Joo et al[100]	Jurgen et al[104]	Current study
I	13.1%	34.4%	27.2%	24%
II	11.4%	16%	13.9%	38%
III	31.1%	31.1%	28.1%	32%
IV	42.6%	18.5%	30.8%	6%

70% cases had lymphatic invasion which was similar to the observation made by Daniela Lazar et al[103], who reported 62.3% and Ji Yoon Choi et al[109] who reported 79.35% cases with lymphatic invasion in his study.

Ji Yoon Choi et al[109] conducted a study in 311 gastric cancer patients and reported vascular invasion in 20.65 % and necrosis in 38.1% of his study population. In comparison with the above mentioned study, this study showed vascular invasion in 26% and necrosis in 34% of the cases.

There were lymphocytic infiltration in 92% and perineural infiltration in 20% of gastric carcinoma cases. This observation is parallel to the 31.7% perineural infiltration reported in the study conducted by Luo Tianhang et al[110].

The expression of p53 and high Ki-67 LI was noted in 64% and 38% cases respectively. This proportion is comparable with the other studies conducted by N.E.Tzanakis et al[102] in the Greek population, Kamran et al[112] in the Arab population, Giovanni et al[107] and Nobuyuki et al[101]. Several studies show p53 expression ranging from 34% to 65%. This fluctuation could be due to different methodologies used and to varying characteristics of the studied cases (Table 47).

TABLE 47 – COMPARISON OF p53 EXPRESSION AND Ki-67 LI IN WORLD STATISTICS

	p53 positive	p53 negative	High Ki-67 LI	Low Ki-67 LI
Y. Maehara et al[111]	38.7%	61.3%	NA	NA
Kamran et al[112]	75%	25%	NA	NA
Zheng et al[113]	53%	47%	93%	7%
Casasola et al[106]	NA	NA	64.9%	35.1%
Nobuyuki et al[101]	58%	42%	42%	58%
Y. Kakeji et al[105]	54.2%	45.8%	NA	NA
Giovanni et al[107]	NA	NA	30.4%	69.6%
Daniela Lazar et al[103]	41%	59%	54.1%	45.9%
Czyzewska et al[108]	NA	NA	64.4%	35.6%
Y.E.Joo et al[100]	34.4%	65.6%	52.1%	47.9%
N.E. Tzanakis et al[102]	65%	35%	46.2%	53.8%
Current study	64%	36%	38%	62%

CORRELATION OF p53 EXPRESSION WITH KNOWN CLINICO-PATHOLOGICAL PROGNOSTIC FACTORS

Y.Kakeji et al (1993) studied 96 gastric carcinomas in Japan and demonstrated statistically significant relationship between p53 expression and lymph node metastasis and vascular invasion and no relationship between stage, size, depth of invasion, grade and histological type[105].

Maehara et al (1999) studied 427 cases of gastric cancer in Japan and demonstrated statistically significant association between p53 expression and tumour size, site, lympho-vascular invasion, lymph node metastasis and stage[111].

Nobuyuki et al (1999) studied p53 expression in 16 early gastric cancers and 15 advanced gastric cancers in Japan and found increased expression of p53 in advanced gastric cancers and in node positive cases[101].

Karman et al (2004) studied 52 gastric cancers in Iran and demonstrated statistically significant association between tumour histologic type, depth of invasion and tumour grade and no significant association with lymph node metastasis[112].

Y.E.Joo et al (2006) studied 119 cases of gastric cancer in Korea and found significant association between p53 expression and depth of invasion. However, there was no association between p53 expression and tumour stage, status of lymph node and survival[100].

N.E.Tzanakis et al (2009) studied 93 gastric cancer patients in Greece and found statistically significant relationship between p53 expression and the tumour size, number of nodes involved, stage and the location of carcinoma within the stomach and no relationship between histological type and grade. Multivariate analysis showed decreased survival (22.3 months) for patients with carcinomas expressing p53 when compared to those not (44months)[102].

Daniela Lazar et al (2010) studied 61 gastric cancer patients in Romania and found statistically significant association between p53 expression and tumour grade, Lauren's histological type, depth of invasion and number of nodes involved and increased survival[103].

In the present study, there was a direct significant association between tumour location and lymphatic invasion with p53 expression. Pan-gastric tumours and tumours of the distal stomach showed significantly increased percentage of cases showing p53 expression. p53 expression was increased in the elderly and there was a slight predominance in males and intestinal type tumours. 100% of mucinous carcinomas and N2 tumours showed p53 expression. p53 positivity was seen to increase with increasing tumour grade, depth of invasion and stage, but statistically significant association could not be ascertained.

In comparison with the above studies, this study also showed no statistically significant association between p53 expression and Borrmann gross type, tumour size, histological type, lymph node metastasis, vascular invasion and perineural infiltration.

CORRELATION OF Ki-67 LABELING INDEX WITH KNOWN CLINICO-PATHOLOGICAL PROGNOSTIC FACTORS

Giovanni de Manzonni et al (1998) studied 56 patients who underwent curative gastrectomy for advanced gastric cancer in Italy and demonstrated no significant association between Ki-67 labeling index and depth of tumour invasion, nodal status and histological type. They also found that the proliferative activity in advanced gastric cancers may not significantly influence survival except in elderly patients[107].

Nobuyuki et al (1999) studied Ki-67LI in 16 cases of early gastric cancer and 15 cases of advanced gastric cancer in Japan and found significantly higher levels of Ki-67 labeling in the advanced gastric cancer group compared to the early gastric cancer group. No significant association was found between Ki-67 LI and lymph node metastasis[101].

Czyzewska et al (2004) studied tumours from 45 gastric cancer patients in Poland and found a statistically significant association between Ki-67 LI and histological type, presence of lymph node metastasis and tumour differentiation and no association was found with depth of invasion and stage[108].

Casasola et al (2004) studied the prognostic value of Ki-67, Cyclin – D1, Cyclin – D3 and CDK4 in 74 cases of gastric cancer. The statistical results showed that only Ki-67 was an independent significant prognostic factor and correlated well with the length of survival[106].

Y.E.Joo et al (2006) studied 119 gastric cancers in Korea and found no association between Ki-67 labeling index and tumour size, depth of invasion, lymph node metastasis, stage and survival. Liu et al[114] and Kanai et al`[115] reported that Ki-67 LI did not influence the prognosis in gastric cancer[100].

N.E. Tzanakis et al (2009) studied 93 gastric cancer cases and found significant association between the Ki-67 LI and lymph node metastasis and stage and no association was found with histological type[102].

Daniela Lazar et al (2010) studied 61 cases of gastric cancer in Romania and demonstrated a statistically significant association between Ki-67 LI and

age, tumour site, histological type and grade. No association was found with lympho-vascular invasion, depth of invasion, lymph node status and stage[116]. The 5-year survival rates of patients with low Ki-67 LI was 17.9% compared to the rate of 15.2% in patients with high LI. This difference in survival rates was not found to be statistically significant[116].

In the present study, there was a direct significant association between Ki-67 LI and perineural infiltration and vascular invasion. Higher Ki-67 LI was seen in mucinous carcinomas and signet ring cell carcinomas and low LI was seen in tubular and papillary carcinomas in concurrence with the prognosis associated with these histological types. A slight predominance of higher LI was seen in males and in intestinal type. A progressive increase in the percentage of cases with high LI was seen with increasing grade, depth of invasion and nodal status which are proven prognostic factors.

In comparison to the above studies, no significant association between Ki-67 LI and age, gender, tumour site, gross type, tumour size, lymphatic invasion and stage was observed.

In the present study when high grade tumours were compared, it was found that the cases with nodal metastasis were positive for p53 immunoreaction but had low Ki-67 LI and the cases with no nodal involvement were p53 negative with high Ki-67 LI. This perhaps indicates that p53 over-expression has more bearing on nodal metastasis than Ki-67 LI.. It was also observed that a few low grade tumours with no nodal metastasis were p53

positive and had a low Ki-67 LI. These tumours require longer follow-up to rule out the possibility of latent nodal metastasis in view of their p53 expression.

TABLE 48 - COMPARISON OF p53 EXPRESSION WITH Ki-67 LI

	Mean Ki-67 LI	
	p53 positive	p53 negative
Y.E.Joo et al [100]	50.7	48.8
Current study	29.362	18.361

Y.E.Joo et al observed in their study that the mean Ki-67 LI value in p53 positive tumours was not significantly higher than that of the p53 negative tumours[100].

However, the present study revealed that the mean Ki-67 LI value in p53 positive tumours (29.362) was significantly (p=0.038) higher than that of p53 negative tumours. P53 and Ki67 were found to be independent prognostic variables with different outcomes for the various clinico-pathological factors.

SUMMARY

SUMMARY

- The percentage of gastric carcinomas among the 9,541 surgical samples received at Madras Medical College in the year 2010 is 3.76%.
- The distribution of non- neoplastic gastric lesions were 45%, benign were 0.6% and malignant tumours were 54.39%.
- Gastric cancers had a peak incidence in the age group of 51 – 60 years.
- 73% cases of gastric cancer were reported in males and 27% in females.
- The most common location of gastric cancer was at the pyloro-antrum which constituted about 60.3% of the cases.
- 58.9% of tumours were less than 5 cm.
- The most common histological type was tubular carcinoma which accounted for 53.9% of cases.
- The most common Lauren's histological subtype was Intestinal carcinoma which accounted for 75.4% of cases.
- G2 (moderately differentiated grade) was the most common grade accounting for 48.7% of cases.
- 46.2% of cases presented in T2 (invasion upto subserosa) and T3 (invasion into the serosa) stage.
- Nodal metastasis was observed in 56.4% of cases.

- Most of the tumours (44.9%) presented in stage II.
- Lymphatic invasion and vascular invasion was seen in 66.6% and19.3% of cases respectively.
- Perineural infiltration was seen in 17.9% of cases.
- Lymphocytic response was seen in 85.8% of cases and necrosis in 23% of cases.
- P53 expression was seen in 64% of cases.
- The mean Ki-67 LI was 25.4%
- High Ki-67 Li was seen in 38% of cases and low LI in 62% of cases.
- p53 expression showed statistically significant association with tumour location and lymphatic invasion.
- An increase in the number of cases with p53 positivity was seen with increasing tumour grade, depth of infiltration, nodal metastasis, stage, mucinous and intestinal type carcinomas.
- No statistically significant association between p53 expression and age, gender, Borrmann gross type, tumour size, vascular invasion, perineural infiltration and necrosis was found.
- There was a direct significant association between Ki-67 LI and perineural infiltration and vascular invasion.

- There was increasing percentage of cases with high Ki-67 LI with increase in the grade, depth of infiltration, nodal metastasis, mucinous, signet ring and intestinal type carcinomas.
- No significant association between Ki-67 LI and age, gender, tumour site, gross type, tumour size, stage, lymphatic invasion and necrosis was found.
- The mean Ki-67 LI value was significantly higher in p53 positive tumours when compared to the LI value in p53 negative tumours.
- No relationship was found between p53 and Ki-67. They were found to be independent prognostic factors.

CONCLUSION

CONCLUSION

The incidence of gastric carcinoma was higher in this study group than the western population. Many patients presented in older age with predominance in males. p53 expression was found in 64% of cases which is similar to that of western population. The mean Ki-67 LI was lower when compared to the other test groups. p53 expression was significantly associated with tumour location and lymphatic invasion. The Ki-67 LI was associated with vascular invasion and perineural infiltration. An increasing percentage of cases with p53 overexpression and high Ki-67 LI was noticed with increasing grade, depth of invasion, nodal status and stage. It was found that the mean Ki-67 LI value was significantly higher in p53 positive cases. It was also noticed that p53 over-expression had more bearing on nodal metastases than Ki-67 LI. Low grade tumours with p53 expression and no nodal metastasis require follow-up to rule out the possibility of latent nodal metastasis in view of their p53 expression.

In conclusion, the role played by cell proliferation in the growth and aggressiveness of gastric tumours is complex and still not clarified. However, identifying the expression of p53 and Ki -67 LI in gastric carcinoma could be helpful to identify a group of patients at high risk of recurrence and poor survival. A larger sample size and follow up of these patients for 5 more years could throw more light on the role of p53 mutation and Ki-67 LI as long term prognostic indicators.

ANNEXURES

ANNEXURE – I

PROFORMA

Case number : Name :

HPE number : Age :

IP number : Sex :

Clinical history :

Risk factors, if any :

Clinical diagnosis :

Imaging :

Endoscopy :

Previous HPE report:

Nature of specimen : Total gastrectomy/Subtotal gastrectomy/Others

GROSS

Proximal circumference : Greater curvature:

Distal circumference : Lesser curvature :

Tumour site :

Tumour size :

Tumour configuration : Depth of invasion:

Margins : Proximal : Distal :

Associated findings :

Total nodes dissected :

MICROSCOPY

Histological type :

Histological grade : G1 / G2 / G4 / G4

Depth of invasion :

Margins : Proximal : Free / Involved

Distal : Free / Involved

Lymphatic invasion : Present / Absent

Venous invasion : Present / Absent

Perineural invasion : Present / Absent

Lymphocytic response : Present / Absent

Necrosis : Present / Absent

Associated findings:

Total number of nodes dissected: Number of nodes involved:

Distant metastasis :

TNM staging :

IMMUNOHISTOCHEMISTRY

P53 score : Intensity –

% of tumour nuclei showing reaction -

Ki67 score: Intensity –

% of tumour nuclei showing reaction -

ANNEXURE - II

WHO CLASSIFICATION OF GASTRIC TUMOURS

EPITHELIAL TUMOURS

Intraepithelial neoplasia – Adenoma

Carcinoma
Adenocarcinoma
- Intestinal type
- Diffuse type

Papillary adenocarcinoma
Tubular adenocarcinoma
Mucinous adenocarcinoma
Signet-ring cell carcinoma
Adenosquamous carcinoma
Squamous cell carcinoma
Small cell carcinoma
Undifferentiated carcinoma
Others
Carcinoid (well differentiated endocrine neoplasm)

NON-EPITHELIAL TUMOURS

Leiomyoma
Schwannoma
Granular cell tumour
Glomus tumour
Leiomyosarcoma
GI stromal tumour
Benign
Uncertain malignant potential
Malignant
Kaposi sarcoma
Others
Malignant lymphomas
Marginal zone B-cell lymphoma of MALT-type
Mantle cell lymphoma
Diffuse large B-cell lymphoma
Others

SECONDARY TUMOURS

ANNEXURE III

TNM STAGING OF GASTRIC TUMOURS

T – Primary Tumour
TX - Primary tumour cannot be assessed
T0 - No evidence of primary tumour
Tis - Carcinoma in situ
T1 - Tumour invades lamina propria or submucosa
T2 - Tumour invades muscularis propria or subserosa
T3 - Tumour penetrates serosa without invasion of adjacent structures
T4 - Tumour invades adjacent structures

N – Regional Lymph Nodes
NX - Regional lymph nodes cannot be assessed
N0 - No regional lymph node metastasis
N1 - Metastasis in 1 to 6 regional lymph nodes
N2 - Metastasis in 7 to 15 regional lymph nodes
N3 - Metastasis in more than 15 regional lymph nodes

M – Distant Metastasis
MX - Distant metastasis cannot be assessed
M0 - No distant metastasis
M1 - Distant metastasis

STAGE GROUPING

Stage 0	Tis	N0	M0
Stage IA	T1	N0	M0
Stage IB	T1	N1	M0
	T2	N0	M0
Stage II	T1	N2	M0
	T2	N1	M0
	T3	N0	M0
Stage IIIA	T2	N2	M0
	T3	N1	M0
	T4	N0	M0
Stage IIIB	T3	N2	M0
Stage IV	T4	N1, N2, N3	M0
	T1, T2, T3	N3	M0
	Any T	Any N	M1

ANNEXURE IV

IMMUNOHISTOCHEMISTRY PROCEDURE

1. 4µ thick sections were cut from formalin fixed paraffin embedded tissue samples and transferred to gelatin-chrome alum coated slides.
2. The slides were incubated at 58ºC for overnight.
3. The sections were deparaffinized in xylene for 15 minutes x 2 changes.
4. The sections were dehydrated with absolute alcohol for 5 minutes x 2 changes.
5. The sections were washed in tap water for 10 minutes.
6. The slides were then immersed in distilled water for 5 minutes.
7. Heat induced antigen retrieval was done with microwave oven in appropriate temperature with appropriate buffer for 20 to 25 minutes.
8. The slides were then cooled to room temperature and washed in running tap water for 5 minutes.
9. The slides were then rinsed in distilled water for 5 minutes.
10. Wash with appropriate wash buffer (citrate buffer) for 5 minutes x 2 changes.
11. Apply peroxidase block over the sections for 10 minutes.
12. Wash the slides in citrate buffer for 5 minutes x 2 changes.
13. Cover the sections with power block for 15 minutes.
14. The sections were drained (without washing) and appropriate primary antibody was applied over the sections and incubated for 1 hour (p53) and 2 hours (Ki-67) respectively.
15. The slides were washed in citrate buffer for 5 minutes x 2 changes.
16. The slides were covered with Super Enhancer for 30 minutes.
17. The slides were washed in citrate buffer for 5 minutes x 2 changes.
18. The slides were covered with SS Label for 30 minutes.
19. Wash in citrate buffer for 5 minutes x 2 changes.
20. DAB substrate was prepared by diluting 1 drop of DAB chromogen to 1 ml of DAB buffer.
21. DAB substrate solution was applied on the sections for 8 minutes.
22. Wash with citrate buffer solution for 5 minutes x 2 changes.
23. The slides are washed well in running tap water for 5 minutes.
24. The sections were counterstained with Hematoxylin stain for 2 seconds (1 dip).
25. The slides are washed in running tap water for 3 minutes.
26. The slides are air dried, cleared with xylene and mounted with DPX.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Parkin DM, Pisani P, Ferlay J. Estimates of the worldwide incidence of 25 major cancers in 1990. Int J Cancer 1999; 80:827-41.
2. Stewart BW, Kleihues P. World Cancer Report. Lyon: IARC Press, 2003
3. Inoue M, Tsugane S. Epidemiology of gastric cancer in Japan. Postgrad Med J 2005; 81:419-24.
4. Bae JM, Jung KW, Won YJ. Estimation of cancer deaths in Korea for the upcoming years. J Korean Med Sci 2002; 17:611-5
5. Blot WJ, Devesa SS, Kneller RW, Fraumeni JF Jr. Rising incidence of adenocarcinoma of the esophagus and gastric cardia. JAMA 1991; 265: 1287-1289
6. Brown LM, Devesa SS. Epidemiologic trends in esophageal and gastric cancer in the United States. Surg Oncol Clin N Am 2002; 11: 235-256
7. Siewert J.R., Sendler A. The current management of gastric cancer. Adv Surg, 1999, 33: 69-93
8. Dicken B. J., Saunders L.D., Jhangri G.S. et al. Gastric cancer: establishing predictors of biologic behavior with use of population based data. Ann Surg Oncol, 2004, 11: 629 – 635

9. Zolota V., Batistatou A., Tsamandas A. C., Iliopoulos G., Scopa C.D., Bonikos D.S. Immunohistochemical expression of TGF-beta1, p21WAF1, p53, Ki-67 and angiogenesis in gastric carcinomas : a clinico-pathologic study. Int J Gastrointest Cancer, 2002, 32: 83 – 89

10. Wong S. K., Seow-Choen F., Leong A. P., Ho Y. H., Aw S. E. Mutant plasma p53 protein levels: prognostication in colorectal carcinoma. Br J Surg, 1997, 84: 664-665.

11. Hashimoto N., Tachibana M., Dhar D. K., Yoshimura H., Nagasue N. Expression of p53 and RB proteins in squamous cell carcinoma of the esophagus : their relationship with clinico-pathologic characteristics. Ann Surg Oncol, 1999, 6: 489-494.

12. Reed W., Hannisdal E., Boehler P. J., Gundersen S., Host H., Marthin J. The prognostic value of p53 and c-erb B-2 immuno -staining is overrated for patients with lymph node negative breast carcinoma: a multivariate analysis of prognostic factors in 613 patients with a follow-up of 14-30 years. Cancer, 2000, 88: 804-813.

13. Maeharay, Tomoda M, Hasuda S. et al. Prognostic value of p53 protein expression for patients with gastric cancer – a multivariate analysis. Br J Cancer, 1999, 79: 1255-1261.

14. Maeharay, Kakeji Y., Watanabe A. et al. Clinical implications of serum anti-p53 antibodies for patients with gastric carcinoma. Cancer, 1999, 85: 302-308.

15. Roviello F., Marrelli D., Vindigni C., De Stefano A., Spina D., Pinto E. P53 accumulation is a prognostic factor in intestinal-type gastric carcinoma but not in the diffuse type. Ann Surg Oncol, 1999, 6: 739-745.

16. Lee K. E., Lee H. J., Kim Y. H. et al. Prognostic significance of p53, nm23, PCNA and c-erbB-2 in gastric cancer. Jpn J Clin Oncol, 2003, 33: 173-179.

17. Lim B. H., Soong R., Grieu F., Robbins P. D., House A. K. Iacopetta B. J. p53 accumulation and mutation are prognostic indicators of poor survival in human gastric carcinoma. Int J Cancer, 1996, 69: 200-204.

18. Brown D. C., Gatter K. C. Monoclonal antibody Ki-67: its use in histopathology. Histopathology, 1990, 17: 489-503.

19. The history of gastric cancer: legends and chronicles : Eugenio Santoro Gastric Cancer (2005) 8: 71–74

20. Pèan JE. De l'ablation des tumeurs de l'estomac par la gastrectomie. Gaz Hop 1879; 52:473–5.

21. Billroth T. Offenes schreiben an Herrn Dr. Wittelshofer. Wien Med Wochenschr 1881; 31:162–5.

22. Lauren P. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. Acta Pathol Microbiol Scand, 1965; 64: 31.

23. Ming SC. Gastric carcinoma: a patho-biological classification. Cancer, 1977; 39: 2475.

24. Oota, K. & Sobin, L.H. (1977). Histological typing of gastric and oesophageal tumors. International Histological Classification of Tumours. n. 18. WHO: Geneva.

25. Yamamoto S. Stomach cancer incidence in the world. Jpn J Clin Oncol 2001; 31: 471

26. Ahn YO, Park BJ, Yoo KY, Kim NK, Heo DS, Lee JK, Ahn HS, Kang DH, Kim H, Lee MS. Incidence estimation of stomach cancer among Koreans. J Korean Med Sci 1991; 6: 7-14

27. Parkin DM. International variation. Oncogene 2004; 23: 6329-6340

28. Keechilat Pavithran, Dinesh C. Doval, and Kamal K. Pandey. Gastric cancer in India. Gastric Cancer (2002) 5: 240–243

29. Malhotra SL. Geographical distribution of gastrointestinal cancers in India with special reference to causation. Gut 1967; 8: 361–72.

30. Ries Lag, Kosary CL, Hankey BF, Miller BA, Harras A, Edwards BK. SEER Cancer Statistics Review 1973-1994, National Cancer Institute,

NIH Publication No. 97-2789. Bethesda: Department of Health and Human Services, 1997

31. Correa P. Human gastric carcinogenesis. A multistep and multifactorial process. Cancer Res, 1992; 52: 6735.

32. Fenoglio-Preiser CM, Noffsinger AE, Belli J, Stemmermann GN. Pathologic and phenotypic features of gastric cancer. Semin Oncol, 1996; 23: 292.

33. Morson BC, Sobin LH, Grundmann E et al. Precancerous conditions and epithelial dysplasia in the stomach. J Clin Pathol, 1980; 33: 711.

34. Hill MJ, Hawksworth G, Tattersall G. Bacteria, nitrosamines and cancer of the stomach. Br J Cancer, 1973; 28: 562.

35. Correa P. A human model for gastric carcinogenesis. Cancer Res, 1988; 48: 3554.

36. Grain S, Haughey B, Marshall J et al. Diet in the epidemiology of gastric cancer. Nutr Cancer, 1990; 13: 19.

37. Ramón JM, Serra L, Cerdó C, Oromi J. Dietary factors and gastric cancer risk: a case controlled study. Cancer, 1993; 71: 1731.

38. Kono S, Hirohata T. Nutrition and stomach cancer. Cancer Causes and Control, 1996; 7: 41.

39. Mirvish SS. Effects of vitamins C and E on N-nitroso compound formation, carcinogenesis and cancer. Cancer, 1986; 58: 1842.

40. Forman D, Newell DG, Fullerton F et al. Association between infection with Helicobacter pylori and risk of gastric cancer: evidence from a prospective investigation. Br Med J, 1991; 302: 1302.

41. Parsonnett J, Friedman GD, Vandersteen DP et al. Helicobacter pylori infection and the risk of gastric carcinoma. N Engl J Med, 1991; 325: 1127.

42. Crabtree JE, Taylor JD, Wyatt JI. Mucosal IgA recognition of Helicobacter pylori 120kDa protein, peptic ulceration and gastric pathology. Lancet, 1991; 338: 332.

43. Warburton VJ, Everett S, Mapstone NP et al. Clinical and histological association of Cag A and Vac A genotypes in Helicobacter pylori gastritis. J Clin Pathol, 1998; 51: 55.

44. Parsonnet J, Friedman GD, Orentreich N, Vogelman H. Risk for gastric cancer in people with Cag A positive or Cag A negative H. pylori infection. Gut, 1997; 40: 297.

45. Wong W-M, Stamp GWH, Elia G et al. Proliferative populations in intestinal metaplasia: evidence of deregulation in Paneth and goblet cells but not endocrine cells. J Pathol, 2000; 190: 107.

46. Keefer LK, Roller PP. N-nitrosation by nitrite ion in neutral and basal medium. Science, 1973; 181: 1245

47. Davies GR, Symmons NJ, Stevens TRJ et al. Helicobacter pylori stimulates antral mucosal reactive oxygen metabolite production in vivo. Gut, 1994; 35: 179.

48. Sobala GM, Crabtree JE, Dixon MF et al. Acute Helicobacter pylori infection: clinical features, local and systemic immune response, gastric mucosal histology and gastric juice ascorbic acid concentration. Gut, 1991; 32: 1415.

49. Guilford P, Hopkins J, Harraway J et al. E-cadherin germline mutations in familial gastric cancer. Nature, 1998; 392: 402.

50. Huntsman DG, Carneiro F, Lewis FR. et al. Early gastric cancer in young, asymptomatic carriers of germ-line E-cadherin mutations. N Engl J Med, 2001; 344: 1904.

51. Park J-G, Park KJ, Ahn Y-O et al. Risk of gastric cancer among Korean familial adenomatous polyposis patients. Dis Colon Rectum, 1992; 35: 996

52. Giardello FM, Welsh SB, Hamilton SR et al. Increased risk of cancer in the Peutz–Jegher syndrome. N Engl J Med, 1987; 316: 511.

53. Kenneth E L McColl, James J Going. Aetiology and classification of adenocarcinoma of the gastro-oesophageal junction/cardia Gut2010;59:282-284 doi:10.1136/gut.2009.186825

54. Murakami T. Patho-morphological diagnosis: definition and growth classification of early gastric cancer. In: Murakami T, ed. Early Cancer. Gann Monograph on Cancer Research 11. Tokyo: University of Tokyo Press, 1971: 53.

55. Stout AP. Superficial spreading type of carcinoma of the stomach. Arch Surg, 1942; 44: 651.

56. Mason MK. Surface carcinoma of the stomach. Gut, 1965; 6: 185.

57. Gutmann RA. De quelques signes radiologiques au cancer gastrique au début. Bull Soc Radiol Med France, 1933; 21: 347.

58. Eckardt VF, Giessler W, Kanzler G et al. Clinical and morphological characteristics of early gastric cancer. A case control study. Gastroenterology, 1990; 98: 708.

59. Everett SM, Axon ATR. Early gastric cancer: disease or pseudo-disease? Lancet, 1998; 351: 1350.

60. Ohta H, Noguchi Y, Takagi K et al. Early gastric carcinoma with special reference to macroscopic classification. Cancer, 1987; 60: 1099.

61. Everett SM, Axon ATR. Early gastric cancer in Europe. Gut, 1997; 41: 142.

62. Dekker W, Tytgat GN. Diagnostic accuracy of fiber-endoscopy in the detection of upper intestinal malignancy. Gastroenterology, 1977; 73: 710.

63. Carneiro F (1997). The distinction between dysplasia and truly invasive cancer. Classification of gastric carcinomas. Curr Diagn Patho l 4: 51-59.

64. Hamilton S R, Aaltonen L A 2000 Pathology and genetics of tumours of the digestive system. World Health Organization Classification of Tumours. Vol 2. IARC Press, Lyon.

65. Takubo K, Honma N, Sawabe M et al. 2002. Oncocytic adenocarcinomas of the stomach: Parietal cell carcinoma. Am J Surg Pathol 26: 458 – 465.

66. Mulligan RM. Histogenesis and biologic behaviour of gastric carcinoma. In: Sommers SC, ed. Gastrointestinal and Hepatic Pathology Decennial 1966–75. New York: Appleton-Century- Crofts, 1975: 31.

67. Goseki N, Takizawa T, Koike M. Differences in the mode of extension of gastric cancer classified by histological type: new histological classification of gastric carcinoma. Gut, 1992; 33: 606.

68. Mori T, Iwashita A, Enjoji M. Adenosquamous carcinoma of the stomach. A clinico-pathologic analysis of 28 cases. Cancer, 1986; 57: 333.

69. Boswell JT, Helwig EB. Squamous cell carcinoma and adenoacanthoma of the stomach. Cancer, 1965; 18: 181.

70. Nagai E, Ueyama T, Yao T, Tsuneyoshi M. Hepatoid adenocarcinoma of the stomach. Cancer, 1993; 72: 1827.

71. Krulewski T, Cohen LB. Choriocarcinoma of the stomach: pathogenesis and clinical characteristics. Am J Gastroenterol, 1988; 83: 1172?

72. Minamoto T, Mai M, Watanabe K et al. Medullary carcinoma with lymphocytic infiltration of the stomach. Cancer, 1990; 66: 945.

73. Rindi G, Bordi C, Rappel S et al. 1996 Gastric carcinoids and neuroendocrine carcinomas: pathogenesis, pathology and behavior. World J Surg 20: 168-172.

74. Capella C, Frigerio B, Cornaggio M. Gastric parietal cell carcinoma—a newly recognised entity: light microscopic and ultra-structural features. Histopathology, 1984; 8: 813.

75. Ueyama T, Nagai E, Yao et al. 1993 Vimentin positive gastric carcinoma with rhabdoid features. Am J Surg Pathol 17: 813-819.

76. Robey-Cafferty SS, Grignon D, Ro J Y et al.1990 Sarcomatoid carcinoma of the stomach. A report of three cases with immunohistochemical & ultrastructural observations. Cancer 65: 1601-1606

77. Esaki Y, Hirayama R, Hirokawa K. A comparison of patterns of metastasis in gastric cancer by histological type and age. Cancer, 1990; 65: 2086.

78. Sobin L H, Wittekind Ch (eds) 2002 UICC. TNM classification of malignant tumours, 6th edition, John Wiley, New York.

79. Nakamura K, Ueyama T, Yao T, Xuan ZX, Ambe K, Adachi Y, Yakeishi Y, Matsukuma A, Enjoji M. Pathology and prognosis of gastric carcinoma. Findings in 10,000 patients who underwent primary gastrectomy. Cancer 1992, 70: 1030-1037.

80. Dupont J B Jr, Lee JR, Burton GR, Cohn I Jr. Adenocarcinoma of the stomach. Review of 1,497 cases. Cancer 1978 41: 941-947.

81. Noguchi Y, Imada T, Matsumoto A, Coit DG, Brennan MF: Radical surgery for gastric cancer. A review of the Japanese experience. Cancer 1989, 64: 2053-2062.

82. Tsukuma H, Oshima A, Narahara H, Morii T. Natural history of early gastric cancer: a non-concurrent long term follow-up study. Gut, 2000; 47: 618.

83. Jakl R, Miholic J, Koller R et al. Prognostic factors in adenocarcinoma of the cardia. Am J Surg, 1995; 169: 316

84. Bowrey DJ, Clark GW, Rees BI et al. Outcome of oesophago-gastric carcinoma in young patients. Postgrad Med J, 1999; 75: 22.

85. Davessar K, Pezzullo JC, Kessimian N, Hale JH, Jauregui HO. Gastric adenocarcinoma. Prognostic significance of several pathological parameters and histological classifications. Hum Pathol 1990, 21: 325-332.

86. Ichikura T, Tomimatsu S, Okusa Y, Uefuji K, Tamakuma S. Comparison of the prognostic significance between the number of metastatic lymph nodes and nodal stage based on their location in patients with gastric cancer. J Clin Oncol 1993 11: 1894-1900.

87. Rugge M, Sonego F, Panozzo M et al. Pathology and ploidy in the prognosis of gastric cancer with no extranodal metastases. Cancer, 1994; 73: 1127.

88. Pinto-De-Sousa J, David L, Almeida R, Leitao D, Preto JR, Seixas M, Pimenta A. c-erb B2 expression is associated with tumour location and venous invasion and influences survival of patients with gastric carcinomas. Int J Surg Pathol 2002, 10:247-256.

89. Joypaul BV, Hopwood D, Newman ELet al. The prognostic significance of the accumulation of p53 tumour-suppressor gene protein in gastric adenocarcinoma. Br J Cancer, 1994; 69: 943.

90. Hurlimann J, Saraga EP. Expression of p53 protein in gastric carcinomas. Am J Surg Pathol, 1994; 18: 1247.

91. Shun CT, Wu MS, Lin JT et al. An immunohistochemical study of E-cadherin expression with correlations to clinico-pathological features in gastric cancer. Hepato-gastroenterology, 1998; 45: 944.

92. Bancroft JD, Marilyn Gamble (Ed), Theory and practice of histological techniques, Churchill Livingstone 2002.

93. Huang S, Minnassian H, More J D et al. Application of immuno-fluorescent staining in paraffin sections improved by trypsin digestion, Laboratory Investigation 35:383-391.

94. Miller K, Auld J, Jessup E, Rhodes A, Antigen unmarking by pressure cooker method. A comparison with microwave oven heating and traditional methods, Advances of anatomical pathology, 2:60-64.

95. Pluzek KY, Sweeney E, Miller KD, Isaacson P, A major advance for IHC Epos, J Pathol 169 (Suppl) abstract 220.

96. Strickler JG, Zheng J, Shu Q et al. p53 mutations and microsatellite instability in sporadic gastric cancer: When guardians fail. Cancer Res, 1994; 54: 4750.

97. Gabbert HE, Muller W, Schneiders A et al. The relationship of p53 expression to the prognosis of 418 patients with gastric carcinoma. Cancer, 1995; 76: 720.

98. Scholzen T, Gerdes J (March 2000). "The Ki-67 protein: from the known and the unknown". J. Cell. Physiol. 182 (3): 311–22.

99. Bullwinkel J, Baron-Lühr B, Lüdemann A, Wohlenberg C, Gerdes J, Scholzen T (March 2006). "Ki-67 protein is associated with ribosomal RNA transcription in quiescent and proliferating cells". J. Cell. Physiol. 206 (3): 624–35.

100. Young-Eun Joo, Ik-Joo Chung, Young-Kyu Park, Yang-Seok Koh, Jae-Hyuk Lee, Chang-Hwan Park, Wan-Sik Lee, Hyun-Soo Kim, Sung-Kyu Choi, Jong-Sun Rew, Chang-Soo Park. (2006) Expression of Cyclooxygenase-2, p53 and Ki-67 in Gastric Cancer. J Korean Med Sci 2006; 21: 871-6.

101. Nobuyuki Igarashi, Makoto Takahashi, Haruo Ohkubo, Kousaku Omata, Ryuichi Iida, Shigeru Fujimoto. Predictive Value of Ki-67, p53 Protein, and DNA Content in the Diagnosis of Gastric Carcinoma. CANCER October 15, 1999 / Volume 86 / Number 8.

102. N.E. Tzanakis, G. Peros, P. Karakitsos, G.A. Giannopoulos, S.P. Efstathiou, G. Rallis, Chr. Tsigris, A. Kostakis, N. I. Nikiteas. (2009) Prognostic Significance of p53 and Ki67 Proteins Expression in Greek Gastric Cancer Patients. Acta Chir Belg, 2009, 109, 606-611.

103. Daniela Lazăr, Sorina Tăban,I. Sporea, Alis Dema, Mărioara Cornian, Elena Lazăr, A. Goldiş, Iulia Raţiu, C. Vernic. (2010) The immunohistochemical expression of the p53-protein in gastric carcinomas. Correlation with clinico-pathological factors and survival of patients. Romanian Journal of Morphology and Embryology 2010, 51(2):249–257

104. Jurgen D. Roder, Knut Bottcher, J. Rudiger Siewert, Raymonde Busch, Tt Paul Hermanek, Hans-Joachim Meyer and the German Gastric Carcinoma Study Group. Prognostic Factors in Gastric Carcinoma. Results of the German Gastric Carcinoma Study 1992

105. Y. Kakeji, D. Korenaga, S. Tsujitani, H. Baba, H. Anai, Y. Maehara & K. Sugimachi . Gastric cancer with p53 overexpression has high potential for metastasizing to lymph nodes. Br. J. Cancer (1993), 67, 589 593.

106. S. Valerdiz Casasola, M. J. Menéndez Colunga, O. Aller Millán, J. M. Martínez Rodríguez. Prognostic value of clinico-pathologic factors Ki67, cyclin D1, cyclin D3 and CDK4 in gastric carcinoma

107. Giovanni de Manzon P, Giuseppe Verlato, Anna Tomezzoll, Alfredo Guglielmi, Giuseppe Pelosi, Francesco Blccl, Alberto Oi Leo and Claudio Cordiano. Study on Ki-67 Immunoreactivity as a Prognostic Indicator in Patients with Advanced Gastric Cancer. Jpn J Clin Oncol1998;28(9)534-537

108. Czyzewska J, Guziñska Ustymowicz K, Lebelt A, Zalewski B, Kemona A. Evaluation of proliferating markers Ki-67, PCNA in gastric cancers. Annales Academiae Medicae Bialostocensis. Vol. 49, 2004 Suppl. 1, Proceedings.

109. Ji Yoon Choi, Tae Kyung Ha, and Sung Joon Kwon. Clinico-pathologic Characteristics of Gastric Cancer Patients according to the Timing of the Recurrence after Curative Surgery. J Gastric Cancer. 2011 March; 11(1): 46–54.

110. Luo Tianhang & Fang Guoen & Bi Jianwei & Ma Liye. The Effect of Perineural Invasion on Overall Survival in Patients with Gastric Carcinoma. J Gastrointest Surg (2008) 12:1263–1267.

111. Y Maehara, M Tomoda, S Hasuda, A Kabashima, E Tokunaga, Y Kakeji and K Sugimachi. Prognostic value of p53 protein expression for patients with gastric cancer - a multivariate analysis. British Journal of Cancer (1999) 79(7/8), 1255–1261.

112. Kamran Ghaffarzadegan , Mohammad-Reza Zali, Khadijeh Jami-Ahmadi Pharm, Hamid Asadzadeh, Mohammad-Reza Abbaszadegan. Correlation of nuclear p53 immunoreaction with the histo-pathologic features in Gastric carcinoma. Arch Iranian Med 2004; 7 (4): 279 – 283.

113. Huachuan Zheng, Koichi Tsuneyama, Chunmei Cheng, Hiroyuki Takahashi, Zhengguo Cui, Yoshihiro Murai, Kazuhiro Nomoto and Yasuo Takano. An Immunohistochemical Study of P53 and Ki-67 in Gastro-intestinal Adenoma and Adenocarcinoma using Tissue Microarray Anticancer research 26: 2353-2360 (2006).

114. Liu XP, Tsushimi K, Tsushimi M, Kawauchi S, Oga A, Furuya T, Sasaki K. Expression of p21 (WAF1/CIP1) and p53 proteins in gastric carcinoma: its relationships with cell proliferation activity and prognosis. Cancer Lett 2001; 170: 183-9.

115. Kanai T, Konno H, Maruyama K, Baba M, Tanaka T, Maruo Y, Nishino N, Nakamura S, Baba S, Sugimura H. p53 overexpression and proliferative activity do not correlate with lymph node metastasis in early gastric cancer. Eur Surg Res 1997; 29: 35-41.

116. Daniela Lazăr, Sorina Tăban, I. Sporea, Alis Dema, Mărioara cornianu, Elena Lazăr, A. Goldiş, Corina vernic. Ki-67 expression in gastric cancer. Results from a prospective study with long-term follow-up. Romanian Journal of Morphology and Embryology 2010, 51(4):655–661.

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S.N	HPEno	Age	Sex	Proc	Site	Gross	Size	Hist. type	Lauren	Grade	Depth	Margins	LI	VI	PNI	Lym	Nec	LN	Stage	P53	Ki67
1.	80/10	46	M	STG	Pyloro – antrum	B – I	3	Diffuse	DT	G3	T3	Free	A	A	A	P	A	N0	II	-	-
2.	110/10	65	F	STG	Pyloro – antrum	B – I	2	Tubular	IT	G2	T2	Free	A	A	A	P	A	N0	IB	Positive	16.4%
3.	151/10	60	F	Pal G	Pyloro – antrum	B – III	6	Tubular	IT	G2	T2	Distal	A	A	A	P	A	N0	IB	Negative	9.3%
4.	207/10	55	M	STG	Pyloro – antrum	B - II	6	Diffuse	DT	G3	T4	Free	P	A	A	P	A	N1	IV	-	-
5.	399/10	59	M	STG	Pyloro – antrum	B – I	4	Mucinous	IT	G2	T3	Free	P	P	A	P	A	N0	II	Positive	56.6%
6.	558/10	60	M	STG	Body	B – III	8	Tubular	IT	G2	T3	Distal	P	P	A	P	A	N1	II	-	-
7.	638/10	58	M	STG	Pyloro – antrum	B – III	5	Papillary	IT	G1	T3	Free	P	P	A	P	P	N1	IIIA	Positive	41.1%
8.	751/10	40	M	STG	Pyloro – antrum	B – II	6	Diffuse	DT	G3	T2	Distal	A	A	A	P	A	N1	II	Positive	18.2%
9.	921/10	58	M	STG	Pyloro – antrum	B – II	5	Tubular	IT	G2	T2	Free	P	P	P	A	P	N1	II	Positive	13.4%
10.	938/10	60	M	TG	OGJ	B – II	5	Tubular	IT	G1	T3	Free	P	A	A	P	A	N1	IIIA	-	-
11.	1238/10	62	M	STG	Pyloro - antrum	B – III	6	Mucinous	IT	G3	T3	Free	P	A	P	P	A	N1	IIIA	-	-
12.	1534/10	64	M	STG	Body	B – II	11	Tubular	IT	G3	T3	Free	P	P	A	P	P	N2	IIIB	Positive	52.3%
13.	1802/10	35	F	STG	Pyloro - antrum	B – III	5	Mucinous	IT	G2	T3	Free	P	P	A	P	A	N1	IIIA	Positive	49.6%
14.	1886/10	55	M	STG	Pyloro – antrum	B – I	3	Mucinous	IT	G3	T3	Distal	P	P	P	P	A	N2	IIIB	Positive	20.1%
15.	2007/10	63	M	STG	Body	B – I	8	Diffuse	DT	G3	T4	Free	P	A	A	P	P	N1	IV	Positive	14.6%
16.	2130/10	65	M	STG	Body	B – III	9	Tubular	IT	G2	T4	Distal	P	P	A	P	A	N2	IV	Positive	15.8%
17.	2190/10	55	M	STG	Body	B – I	10	Papillary	IT	G1	T3	Free	P	P	A	P	P	N0	II	Negative	17.6%
18.	2456/10	47	F	STG	Pyloro - antrum	B – II	5	Diffuse	DT	G3	T2	Free	A	A	A	A	A	N0	IB	-	-
19.	2648/10	60	M	STG	Pyloro – antrum	B – III	3	Tubular	IT	G2	T2	Free	A	A	A	P	A	N0	IB	Negative	14.1%
20.	2678/10	70	M	TG	OGJ	B – II	3	Tubular	IT	G2	T3	Proximal	A	A	A	P	A	N0	II	Negative	13.5%
21.	2906/10	33	M	TG	Diffuse	B – IV	-	Diffuse	DT	G3	T3	Free	P	A	A	P	A	N1	IIIA	Negative	6.9%
22.	2922/10	50	M	TG	Diffuse	B – IV	-	Diffuse	DT	G3	T3	Both	A	A	A	P	A	N0	II	Positive	18.4%
23.	3194/10	40	F	STG	Fundus	B – II	6	Tubular	IT	G2	T2	Free	P	A	A	P	A	N1	II	Negative	6.2%
24.	3418/10	62	M	STG	Pyloro – antrum	B – III	6	Tubular	IT	G2	T2	Free	P	A	A	P	A	N0	IB	Positive	5.4%
25.	3551/10	52	F	STG	Pyloro – antrum	B – III	5	Diffuse	DT	G3	T3	Free	A	A	A	P	A	N0	II	-	-
26.	3584/10	53	M	STG	Pyloro – antrum	B - IV	2	Tubular	IT	G2	T2	Free	A	A	A	P	A	N0	IB	Positive	38.8%
27.	3667/10	52	M	STG	Pyloro - antrum	B – II	3	Tubular	IT	G2	T2	Free	A	A	A	P	A	N0	IB	Negative	10.5%
28.	3702/10	73	M	TG	Diffuse	B – IV	-	Mucinous	IT	G2	T3	Proximal	P	A	A	P	A	N1	IIIA	Positive	11.6%
29.	3729/10	35	F	TG	OGJ	B – II	2	Tubular	IT	G2	T4	Proximal	P	A	A	A	A	N1	IV	-	-

30.	3912/10	65	F	TG	Pyloro - antrum	B - II	5	Tubular	IT	G2	T2	Free	P	A	A	P	A	N1	II	-	-
S.n	HPE no	Age	Sex	Proc	Site	Gross	Size	Hist. type	Lauren	Grade	Depth	Margins	LI	VI	PNI	Lym	Nec	LN	Stage	P53	Ki67
31.	4002/10	43	M	STG	Pyloro - antrum	B - IV	1.5	Diffuse	DT	G3	T2	Distal	A	A	P	A	A	N0	IB	-	-
32.	4412/10	58	M	STG	Pyloro – antrum	B – III	4.5	Tubular	IT	G1	T3	Free	P	A	A	P	A	N0	II	Positive	9.2%
33.	4501/10	45	M	Pal G	Pyloro - antrum	B – I	3	Diffuse	DT	G3	T4	Free	A	A	A	P	A	N0	IV	Negative	50.2%
34.	4691/10	35	F	TG	Diffuse	B – IV	-	SIgnet	DT	G3	T3	Free	P	A	A	P	A	N1	IIIA	Positive	18.3%
35.	4821/10	62	F	STG	Pyloro - antrum	B – II	3	Tubular	IT	G3	T3	Distal	P	A	A	A	P	N1	IIIA	Positive	69.3%
36.	4983/10	58	M	STG	Pyloro - antrum	B – II	3.5	Tubular	IT	G2	T2	Distal	P	P	P	P	A	N1	II	Positive	25.5%
37.	4987/10	50	F	STG	Pyloro - antrum	B – II	6	Mucinous	IT	G2	T2	Free	P	A	A	P	A	N1	II	-	-
38.	5123/10	62	M	TG	Fundus	B – II	12	Tubular	IT	G3	T2	Proximal	P	A	P	P	P	N0	II	Positive	27.8%
39.	5205/10	62	F	TG	OGJ	B – II	1.5	Tubular	IT	G1	T2	Free	A	A	A	P	A	N1	II	Negative	8.1%
40.	5315/10	50	M	STG	Pyloro - antrum	B - III	4	Mucinous	IT	G2	T2	Free	P	A	A	P	A	N1	II	-	-
41.	5376/10	39	M	STG	Pyloro - antrum	B – II	4.5	Tubular	IT	G2	T2	Free	A	A	A	A	A	N0	IB	-	-
42.	5445/10	50	M	STG	Pyloro - antrum	B – II	5	Mucinous	IT	G2	T2	Free	A	A	A	P	P	N0	IB	-	-
43.	5489/10	73	M	STG	Pyloro - antrum	B - III	3	Tubular	IT	G2	T2	Free	P	A	A	P	A	N0	II	-	-
44.	5716/10	54	M	STG	Body	B – II	2	Tubular	IT	G3	T2	Free	P	A	A	P	P	N1	II	Positive	17.3%
45.	5743/10	50	F	STG	Body	EGC - III	4	Signet	DT	G3	T1	Free	A	A	A	P	A	N0	IA	-	-
46.	5804/10	48	M	STG	Pyloro - antrum	B – II	6.5	Mucinous	IT	G2	T3	Free	P	A	A	P	A	N2	IIIB	Positive	25.7%
47.	5933/10	28	F	TG	OGJ	B – I	6	Signet	DT	G3	T2	Proximal	P	P	P	P	A	N0	IB	Negative	28.3%
48.	6093/10	59	M	STG	Body	B – III	5	Tubular	IT	G2	T2	Free	P	A	P	P	A	N1	II	-	-
49.	6320/10	65	M	STG	Pyloro - antrum	B – I	4	Papillary	IT	G1	T3	Distal	P	A	A	P	A	N1	IIIa	-	-
50.	6331/10	50	F	TG	OGJ	B – III	6	Tubular	IT	G2	T2	Proximal	A	A	A	P	A	N0	IB	Negative	7.1%
51.	6372/10	23	F	STG	Pyloro - antrum	B – III	3	Tubular	IT	G2	T2	Free	P	A	A	P	A	N1	II	-	-
52.	6454/10	60	M	STG	Pyloro - antrum	B – III	6	Tubular	IT	G3	T3	Distal	P	A	A	P	P	N1	IIIA	Positive	16.1%
53.	6486/10	75	M	STG	Body	B - III	5	Mucinous	IT	G2	T2	Distal	P	A	P	P	A	N0	IB	Positive	44.8%
54.	6503/10	73	M	STG	Pyloro - antrum	B - IV	3	Tubular	IT	G2	T3	Free	P	A	A	P	A	N0	II	Positive	10.2%
55.	6597/10	37	M	STG	Pyloro - antrum	B – II	3	Tubular	IT	G2	T2	Free	A	A	A	P	A	N0	IB	Negative	43.1%
56.	6723/10	75	M	STG	Pyloro - antrum	B – II	6	Signet	DT	G3	T3	Free	P	P	P	P	A	N1	IIIA	-	-
57.	6900/10	80	M	STG	Pyloro - antrum	B - III	14	Tubular	IT	G2	T3	Free	P	A	A	P	A	N0	II	-	-
58.	7127/10	60	M	STG	Pyloro - antrum	B – I	4	Tubular	IT	G1	T3	Free	P	A	A	P	A	N1	IIIA	Positive	24.8%

59.	7165/10	60	M	STG	Body	B – II	6	Papillary	IT	G1	T2	Free	P	A	A	P	P	N1	II	Negative	9.8%
60.	7176/10	50	M	STG	Body	B - IV	5	Tubular	IT	G3	T3	Free	A	A	A	P	A	N0	II	-	-
S.n	HPEno	Age	Sex	Proc	Site	Gross	Size	Hist. type	Lauren	Grade	Depth	Margins	LI	VI	PNI	Lym	Nec	LN	Stage	P53	Ki67
61.	7239/10	35	M	TG	Body	B – III	5	Signet	DT	G3	T3	Distal	P	P	P	P	A	N1	IIIA	Negative	37.2%
62.	7306/10	64	F	STG	Pyloro - antrum	B - II	8	Diffuse	DT	G3	T2	Free	P	A	A	P	A	N1	II	-	-
63.	7830/10	65	F	TG	OGJ	B - III	6	Diffuse	DT	G3	T2	Free	P	A	A	A	A	N1	II	-	-
64.	8338/10	50	M	STG	Pyloro - antrum	B – II	6	Tubular	IT	G1	T2	Free	A	A	A	P	P	N0	IB	Positive	4.9%
65.	8586/10	49	F	TG	OGJ	B – II	4	SCC	-	G2	T2	Proximal	A	A	A	A	A	N1	II	-	-
66.	8612/10	50	M	STG	Body	B – II	3	Tubular	IT	G1	T2	Free	A	A	A	A	A	N1	II	Negative	3.9%
67.	8755/10	55	M	STG	Pyloro - antrum	B – I	2	Tubular	IT	G2	T2	Free	P	P	A	P	P	N1	II	Positive	64.5%
68.	8808/10	60	M	PSTG	Body	B – III	4	Tubular	IT	G2	T3	Distal	P	P	P	P	A	N1	IIIA	Positive	48.7%
69.	8829/10	65	F	STG	Pyloro - antrum	B – II	5	Tubular	IT	G2	T3	Free	P	A	P	P	A	N2	IIIB	Positive	37.6%
70.	9004/10	55	M	TG	OGJ	B – I	9	Tubular	IT	G2	T3	Free	P	A	A	P	P	N1	IIIA	Negative	19.3%
71.	9011/10	45	F	STG	Pyloro - antrum	B – II	5	Diffuse	DT	G3	T2	Free	P	A	A	A	A	N0	IB	Positive	23.4%
72.	9154/10	58	M	STG	Pyloro - antrum	B – II	3	Tubular	IT	G2	T3	Free	A	A	A	P	A	N0	II	-	-
73.	9180/10	45	M	STG	Pyloro - antrum	B – III	2.5	Signet	DT	G3	T3	Free	A	A	A	P	A	N0	II	-	-
74.	9300/10	65	M	STG	Pyloro - antrum	B – II	9	Tubular	IT	G2	T3	Free	P	A	A	P	P	N1	IIIA	Negative	26.1%
75.	9316/10	55	M	STG	Pyloro - antrum	B – II	8	Papillary	IT	G1	T2	Free	P	A	A	P	P	N1	II	Negative	19.3%
76.	9332/10	65	M	TG	Body	B – III	8	Tubular	IT	G3	T3	Free	A	A	A	P	P	N0	II	Positive	75.3%
77.	9400/10	55	M	STG	Body	B – III	2	Tubular	IT	G2	T3	Proximal	P	A	P	P	P	N0	II	Positive	23.9%
78.	9437/10	65	M	STG	Pyloro - antrum	B – III	8	Mucinous	IT	G3	T3	Free	P	A	A	A	A	N1	IIIA	-	-

KEY TO MASTERCHART

Proc – Procedure

Hist. – Histological

LI – Lymphatic invasion

VI – Vascular invasion

PNI – Perineural invasion

Lym – Lymphocytic response

Nec – Necrosis

LN – Lymph Node status

STG – Sub-Total Gastrectomy

Pal G – Palliative Gastrectomy

TG – Total Gastrectomy

OGJ – Oesophago-Gastric Junction

B – Borrmann gross type

EGC – Early Gastric Cancer

IT – Intestinal Type

DT – Diffuse Type

G – Grade

T – Tumour depth

P - Present

A – Absent

N - Node

INSTITUTIONAL ETHICAL COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI -3

Telephone No: 04425305301
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CERTIFICATE OF APPROVAL

To
Dr. Hema Vaneeswari .C
PG in MD Pathology
Madras Medical College, Chennai -3

Dear Dr. Hema Vaneeswari .C

The Institutional Ethical Committee of Madras Medical College reviewed and discussed your application for approval of the project / proposal / clinical trail entitled **"The study of the expression of p53 and Ki-67 Gastric carcinomas and their correlation with clinico pathological variables" No 23072010**

The following members of Ethical committee were present in the meeting held on 21.07.2010 conducted at Madras Medical College,

1. Prof. S.K. Rajan, MD	-- Chairperson
2. Prof. J. Mohanasundaram, MD,Ph.D,DNB Dean, Madras Medical College, Chennai -3	-- Deputy Chairman
3. Prof. A. Sundaram, MD Vice Principal , MMC, Chennai -3	-- Member Secretary
4. Prof. R. Sathianathan, MD Director, Institute of Psychiatry	-- Member
5. Prof R. Nandhini, MD Director, Institute of Pharmacology, MMC, Ch-3	-- Member
6. Prof. Pregna B. Dolia , MD Director, Institute of Biochemistry, MMC, Ch-3	-- Member
7. Prof. C. Rajendran , MD Director, Institute of Internal Medicine, MMC, Ch-3	-- Member -- Member
8. Prof. Geetha Subramanian, MD,DM Professor & Head , Dept. Of Cardiology	-- Member
9. Prof. V. Shruti Kamal, MS Professor of Surgery, MMC, Ch-3	-- Member
10. Prof. Md. Ali, MD, DM Professor & Head ,,Dept. of MGE, MMC, Ch-3	-- Member -- Member

We approve the trail to be conducted in its presented form.

Sd /. Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, any SAE occurring in the course of the study, any changes in the protocol and patient information / informed consent and asks to be provided a copy of the final report

Member Secretary, Ethics Committee.

ABSTRACT

AIM:

The variable prognosis of gastric cancer within a pathological stage necessitates the identificationof subgroups of patients with a more aggressive disease. The role of p53 and Ki67 expression in gastric carcinoma is far from being fully established. The aim of the present study was to identify the incidence and distribution of gastric carcinoma in patients admitted in the Government General Hospital, Chennai in the year 2010 and to evaluate the expression of p53 and Ki67 in gastric cancer and correlate the findings with several clinico-pathological features and prognosis.

MATERIALS AND METHODS:

Formalin-fixed paraffin-embedded tissue samples from 50 patients treated by gastric resection for gastric carcinoma in the year 2010 were studied by immunohistochemistry, using monoclonal antibodies to p53 and Ki67. The results were correlated with clinico-pathological features.

RESULTS:

p53 over-expression was significantly related with tumour location and lymphatic invasion. Higher Ki-67 labeling index correlated significantly with vascular invasion and perineural infiltration. Increasing p53 expression and Ki-67 labeling index was associated with increasinggrade, depth of infiltration, nodal stage and TNM stage. The mean Ki-67 labeling index was higher in p53 positive cases.p53 and Ki-67 were identified as independent prognostic factors.

CONCLUSION:

The role played by cell proliferation in the growth and aggressiveness of gastric tumours is complex and still not clarified. Identifying the overexpression of p53 and Ki -67 LI in gastric carcinoma could be useful as independent prognostic markers in identification of patients at high risk of recurrence and poor survival. Follow up of these patients for 5 more years could throw more light on the role of p53 mutation and Ki-67 LI as long term prognostic indicators.

KEYWORDS: Gastric carcinoma, p53, Ki-67